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THE EDITOR'S VIEWPOINT

Miller, Soberman, and Stahl (p. 4) examined the oral mucosa of young men by the Papanicolaou technic. They found a normal base line of cornification with keratinization most marked on the gingiva, followed by the dorsum of the tongue, the cheek, and the ventrum of the tongue. Montgomery (p. 12) made a similar survey of individuals of different ages and sex. He investigated six areas and found cornification greatest in the gingiva, followed by the dorsum of the tongue, vestibule, soft palate, and cheek. He also investigated the effect of age, sex, and menstrual cycle but found no correlation between any of these and the exfoliative cytology. These two interesting studies compliment each other and offer a valuable basis for further studies of abnormal states. It would be interesting to follow the future oral changes in the subjects that Montgomery reported as deviating markedly from the averages.

Sud's (p. 19) observations on the periodontal condition of 248 malnourished Indian refugees supplies interesting data. The higher degree of occurrence of gingival disease among frankly nutritionally deficient and among pregnant women suggests that systemic factors are important etiologic factors in gingival disease. Local irritants accentuated the disease process. As might be expected, the grossly nutritionally deficient pregnant women had the most severe periodontal changes.

Cabrini and Carranza (p. 28) have confirmed the findings of Zander and of Valloton that phosphatase activity in the gingiva is primarily in the vessels and collagenous fibers. The concentration was greatest in the corium of the epithelial attachment. In the epithelium, the phosphatase was chiefly in the basal layers.

English and Tullis (p. 33) report the oral effects in swine exposed to heavy total body x-ray irradiation. The oral mucosal lesions resembled those common to the alimentary tract, a finding not limited to changes produced by irradiation. Reduction in lymphocytes and granulocytes alters the usual pathologic response and the restoration of function and cells is further hindered by vascular changes. These finding suggest the importance of protecting the oral mucosa from irradiation and from traumatic injury in postirradiation patients. In developing teeth, injury to the ameloblasts may result in hypoplasia.

Afonsky (p. 53) observed the occurrence of dental caries in Central China. He found that the diet of the people in Central China, although rich in carbohydrates, does not predispose to dental caries. The carbohydrate consumed in Central China, Afonsky points out, is chiefly in the form of rice. He suggests that the lack of easily fermentable carbohydrates and sticky foods, and the absence of eating between meals, may be major factors in resistance to dental caries. Afonsky's study supports the belief that adequate diet is not essential for immunity to dental caries.

Wheatcroft, Gerende, Schlack, Taylor, Berzinskas, and Mullins (p. 62) observed the distribution of caries in the rats at the Naval Medical Research Institute and analyzed the results in relationship to bilateral occurrence of lesions. Their studies show that, while bilateral symmetry is high in groups of animals, there may be considerable asymmetry of caries in individuals and that conclusions based on assumption of perfect symmetry may be materially distorted. The average incidence of caries was shown to vary from groove to groove. What differences might be found by subjecting data from experimental caries studies to similar critical statistical analysis is problematical. Certainly, in view of the readiness of many to use methods on the basis of results from small groups studied by unilateral application of chemicals, the analysis of the Navy group is important even though conducted on rats rather than on material from human beings.

Palmer, Overstreet, and Sacks (p. 75) have attempted to evaluate caries control methods by in vitro studies. In effect,

teeth treated by various technics were immersed in acids produced by lactobacilli and the amount of decalcification was determined by weight loss. Such in vitro studies have limited information that may offer important clues, but they should not be considered as replacing in vivo studies with all their complicating factors.

Turner, Crowell, and Dexter (p. 82) have isolated a virus from carious dentin and have been able to propagate it and have demonstrated that it is associated with destruction of rat dentin in vitro. The full implication of the isolation of this virus cannot yet be given but further studies will be necessary to evaluate this process. The questions that might be asked are: the type of cell in which this virus lives in the carious dentin and whether it is capable of existence in dentin or whether it is necessary for it to live in saliva. Certainly, the possible role of a virus in dental caries should not be overlooked.

Ennever, Robinson, and Kitchin (p. 88) discuss actinomycetes and the dentobacterial plaque. These filamentous organisms appear to have an important role in the plaque and thus in the caries process. Many other organisms are encountered in the plaque, and segment by segment we may have portions of this microbial flora investigated in various laboratories to finally give the basis for a thorough understanding of the whole. At that time, the interrelationships of the parts can be better understood.

Coolidge (p. 97), using a method which allows analysis of very small quantities of tissue, investigated the phosphorus and nitrogen content of normal enamel and of enamel from early smooth surface caries. No large differences in the phosphorus or nitrogen content of the normal and carious tissue was observed. There were larger variations in the nitrogen and phosphorus content of enamel from different teeth and from different areas of the same tooth. This method will be useful to biochemists who are investigating the organic structure of normal and pathologic areas of teeth in reference to various pathologic conditions.

Neuwirth and Summerson (p. 100), investigating the action of saliva on glucose, found that as much as 80 per cent of added 0.13 per cent glucose disappeared in 30 minutes. Although lactic acid is a major product of the action of oral microorganisms on glucose and pyruvic acid is occasionally formed, the remaining one-third to one-half of the acid produced is unknown. Caries activity did not affect the acid production at the levels of measurement employed. The production and utilization of acids by the enzyme systems of the oral flora presents an important phase of our knowledge of the caries process. Certainly none of the lactobacillus counts given was significantly high and, as judged by usual standards, all the subjects given in Table II were relatively "caries immune." The influence of different types of flora should be considered as well as the effect of high and low salivary lactobacillus counts.

When penicillin is suggested for use intraorally, the question of production of penicillin-resistant strains of organisms is often raised. Too few studies and too much alarm appears to be the general trend. Lind and Zander (p. 112) found that the daily use of penicillin in a dentifrice for two years did not induce the proliferation of penicillin-resistant strains of streptococci and staphylococci in the throats of children. Certainly this must be considered as a severe test.

Shiere, Georgi, and Ireland (p. 116) report on *Streptococcus salivarius* in its possible relationship to dental caries. They found the organism a normal inhabitant of saliva, increased in salivas of patients with open carious lesions, capable of lowering the pH of media and etching enamel in vitro, and inhibited by sodium fluoride. On the basis of these findings they conclude that it may be an etiologic factor in caries. There appears to be an impression that many caries investigators claim that lactobacilli are the sole acidogenic organisms in dental caries. A review of the literature does not justify this assumption. Lactobacilli are frequently cited as indicators of caries activity and investigated as representative acidogens in antidegredation studies. It is highly probable that many organisms can act as the acidogens in the mouth and that methods controlling one may control many or all oral acidogens.

Haggard and Greenberg (p. 126) found that ingestion of acidulated beverages did not appreciably affect the pH of the saliva and, therefore, should not be considered as a cause of loss of tooth substance when consumed in usual amounts. While their findings appear well founded, it would be a gross misuse of this data to claim that they show that consumption of acidulated beverages does not cause dental caries. Haggard and Greenberg made no effort to study the effect of the sugars in the beverages as etiologic factors in dental caries. As the authors point out, the findings only show that "A corrosive action, so as to cause dental caries, . . . by the *acidity* of fruit juices or soft drinks, appears unlikely in the normal consumption of these beverages." We believe that a decalcifying action is likely if acidogens act on the carbohydrates in these beverages.

Schneyer (p. 130) found that the amylolytic activity of salivary amylase increases with a rise in temperature to 50° C. but falls off beyond this. The high heat of activation indicates that this is a denaturation and one stage is readily reversible. The denaturation is slowed by substrate and by sodium chloride. The amylolytic activity may be of great importance in caries resistance.

Zander, Lisanti, and Shiere (p. 139) brushed hamsters' teeth with antibiotic-containing dentifrices and found results on these animals similar to those on human subjects. From this, they conclude that the hamster is a useful animal for pilot studies in caries investigations. It is also significant that penicillin was most effective of the three antibiotics tested and that there was an optimal level for penicillin concentration in the dentifrice.

Manly, Baker, Miller, and Welch (p. 145) approached the problem of improving silicate cements by changing the modifier in the liquid, by varying the flux, and by using different proportions of oxides in the powder. No general improvement was observed over commercial cement materials distributed to dentists. The basic information derived is interesting. Since much of the work of improving these cements has been conducted as industrial research and the information obtained has not been generally available, this contribution is enlightening.

H. B. G. R.

A STUDY OF THE CORNIFICATION OF THE ORAL MUCOSA OF YOUNG MALE ADULTS

SAMUEL CHARLES MILLER, D.D.S., F.A.C.D., ALEXANDER SOBERMAN, A.B., D.D.S., AND S. SIGMUND STAHL, M.S., D.D.S.

New York University College of Dentistry, New York, N. Y.

THIS study was undertaken for the purpose of comparing the degree of cornification of the oral epithelium, utilizing the technics and findings of Papanicolaou, Traut, and Marchetti.[1] Selected sites of the oral mucosa of healthy young men were chosen for the cytologic examination of the exfoliated cells.

In reviewing the literature on exfoliative cytology, we find that Schroeder[2] in 1915 attempted to correlate ovarian function with cyclic changes in the endometrium. Stockard and Papanicolaou[3] followed this with a study of the estrus cycle of the guinea pig. As an outgrowth of these and other investigations, Papanicolaou[4] in 1933 described the cyclic changes in women as they are reflected in the cellular make-up of the vaginal fluid. For this study, he developed a polychromatic stain which demonstrates by changes and variations in color the degree of cornification of the cell. Papanicolaou[4] stated, "Cornified cells take an intense eosin color and are sharply differentiated. On the other hand, cells derived from the deeper vaginal layers which are free from cornification are stained strongly blue."

The use of exfoliated cells in the study of keratinization of the oral mucosa was first undertaken in 1940 by Weinmann[5] who, using the Wright and Gram stains, reported different degrees of cornification in various sites of the oral mucosa. A comparison of oral and vaginal epithelial smears was made in 1948 by Ziskind and Moulton[6] who used the Shorr modification of the Papanicolaou stain. They observed that cyclic changes occur in the oral epithelium similar to those found in the vagina. An attempt was also made to use the Papanicolaou technic in a study of the oral mucosa by Montgomery[7] in 1949.

MATERIALS AND METHOD

Fifty men students of New York University College of Dentistry, ranging in age from 20 to 30 years, were chosen at random for this investigation.

For a comparison of the degree of cornification, the following four sites were selected: (a) the midline of the anterior third of the dorsal aspect of the tongue; (b) the midline of the anterior third of the ventral aspect of the tongue;

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(c) the area of the right cheek just anterior to the opening of Stenson's duct, and (d) the labial margin of the lower anterior gingivae. In all of the cases, only clinically normal appearing sites were chosen and all of the smears were taken during the forenoon.

Scrapings were taken from each of these selected sites by means of a wooden spatula. These scrapings were transferred to a glass slide which was immediately immersed in equal parts of ether and 95 per cent ethyl alcohol for fixation. The slides were then stained according to the method of Papanicolaou.[1]

A random cell count of 100 cells was made on each slide and the cells found were classified into the following 3 groups: (1) red staining cells with nuclei; (2) blue staining cells with nuclei, and (3) yellow staining cells either with small pyknotic nuclei or anuclear.

FINDINGS

The dorsal and ventral surfaces of the tongue and the buccal mucosa showed essentially two cell types. One, a large, red staining cell with an irregular outline and a small, dense, somewhat pyknotic nucleus. In many cases the nucleus showed definite evidence of undergoing disintegration. The cytoplasm was granular in appearance with folding at the periphery. The blue staining cell was more regular in outline with a somewhat larger, granular, lighter staining nucleus.

In the gingivae, a third distinct cell type was differentiated. This cell stained yellow, often had an indefinite outline, and in the majority of cases showed a complete loss of nuclear elements.

In all of the cases, variations in the color and in the intensity of stain of these cell types were noted; but for the purpose of clarity they were disregarded.

The following statistical findings are reported:

1. *Dorsal Aspect of the Tongue* (Fig. 1)

   Red staining cells: Average number = 86
   Standard deviation = 9.3
   Range ratio = 76.7 - 95.3

   Blue staining cells: Average number = 14
   Standard deviation = 9.1
   Range ratio = 4.9 - 23.1

2. *Ventral Aspect of the Tongue* (Fig. 2)

   Red staining cells: Average number = 21
   Standard deviation = 11.1
   Range ratio = 9.9 - 32.1

   Blue staining cells: Average number = 79
   Standard deviation = 10.9
   Range ratio = 68.1 - 89.9

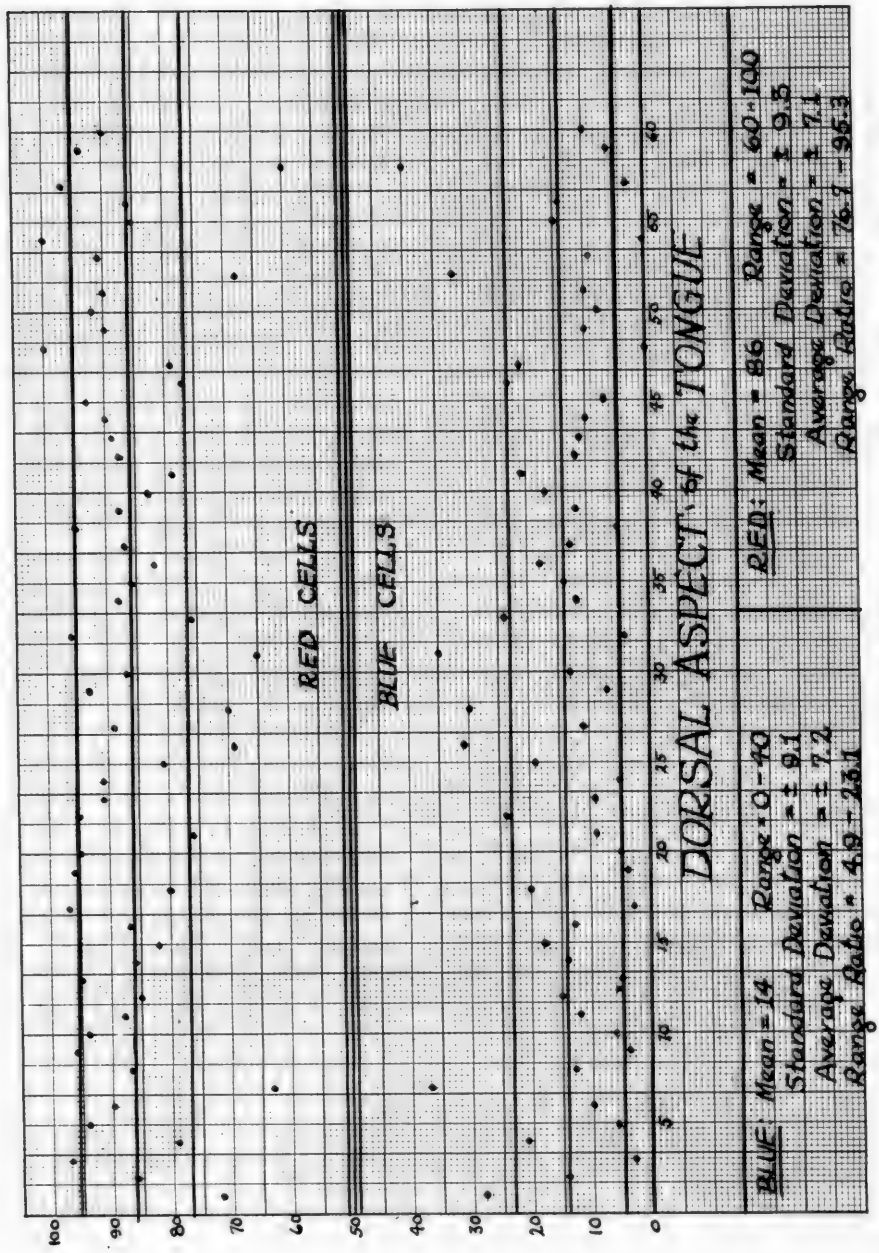
RED CELLS
BLUE CELLS
DORSAL ASPECT of the TONGUE
BLUE: Mean = 14 Range = 0-40
Standard Deviation = ± 9.1
Average Deviation = ± 7.2
Range Ratio = 4.9 - 23.1
RED: Mean = 86 Range = 60-100
Standard Deviation = ± 9.5
Average Deviation = ± 7.4
Range Ratio = 76.7 - 96.3

FIG. 1.

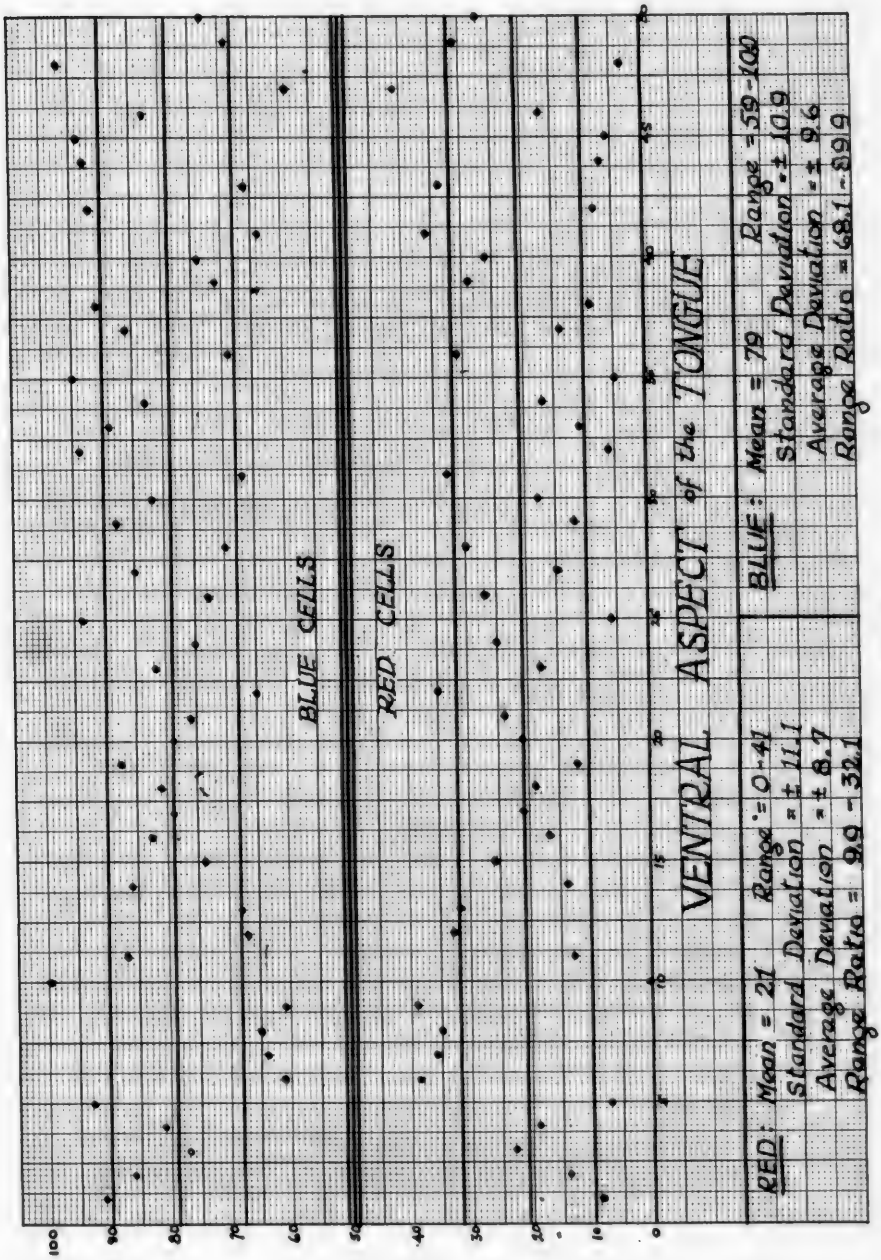
VENTRAL ASPECT of the TONGUE
BLUE CELLS
RED CELLS
RED: Mean = 21 Range = 0-41
Standard Deviation = ± 11.1
Average Deviation = ± 8.7
Range Ratio = 9.9 - 32.1
BLUE: Mean = 79 Range = 59-100
Standard Deviation = ± 10.9
Average Deviation = ± 9.6
Range Ratio = 68.1 - 89.9

Fig. 2.

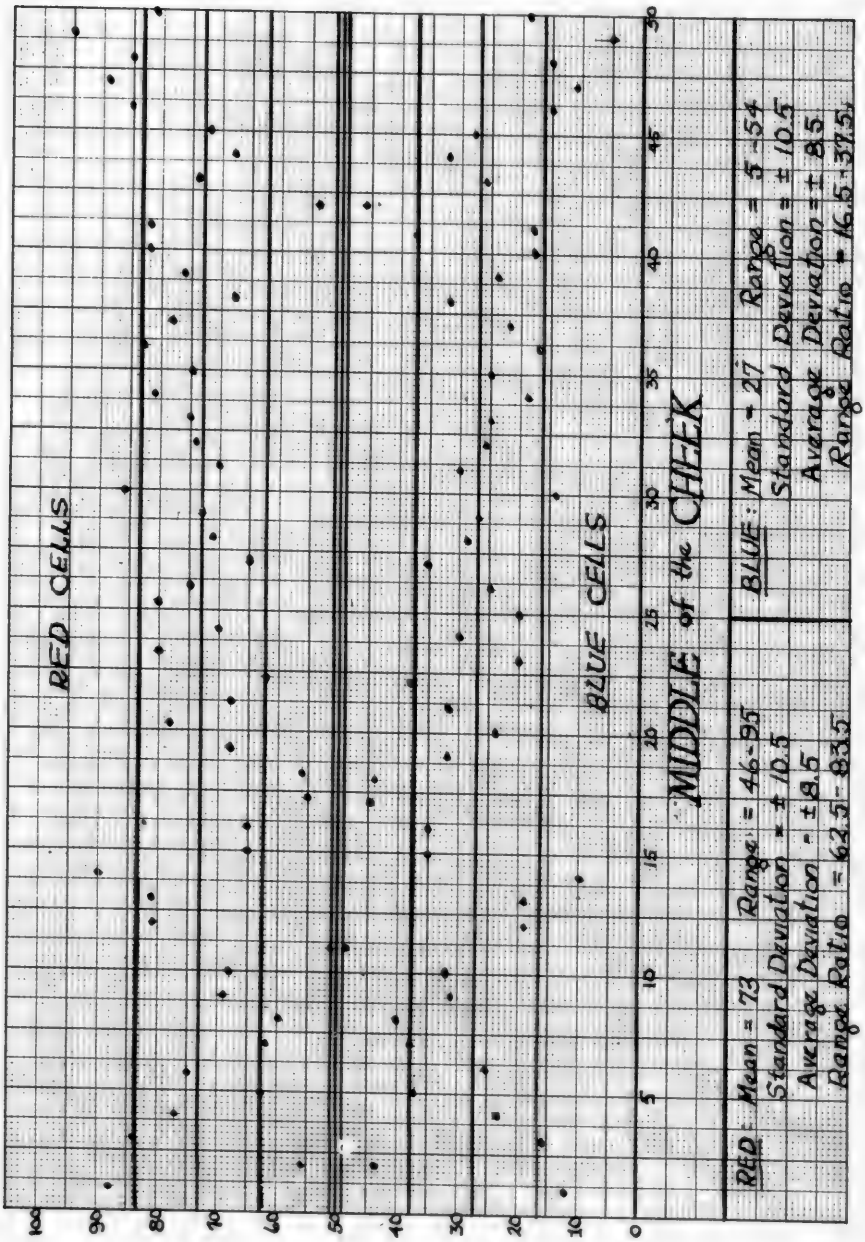
RED CELLS
BLUE CELLS
MIDDLE of the CHEEK
RED: Mean = 73 Range = 46-95
Standard Deviation = ±10.5
Average Deviation = ±8.5
Range Ratio = 62.5-83.5
BLUE: Mean = 27 Range = 5-54
Standard Deviation = ±10.5
Average Deviation = ±8.5
Range Ratio = 16.5-37.5

Fig. 3.

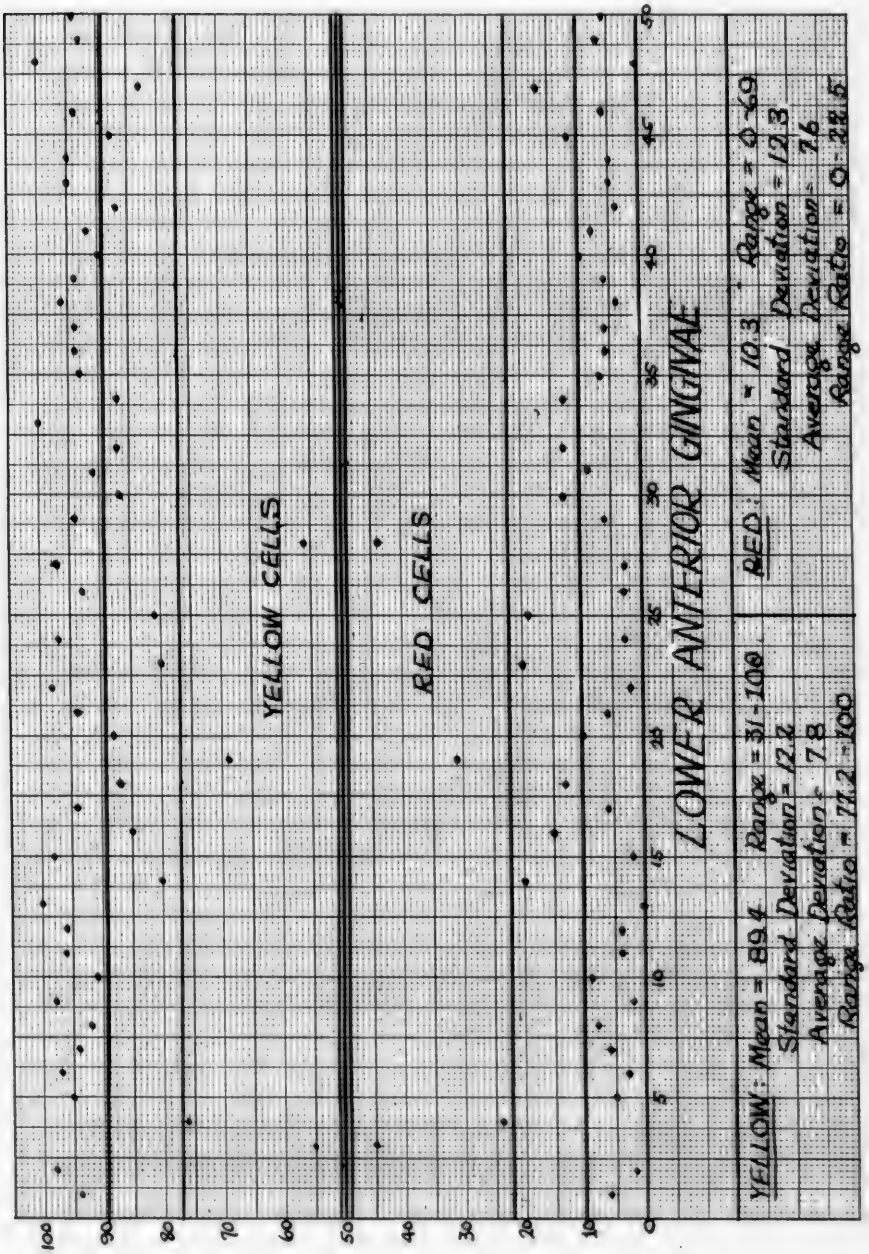
YELLOW CELLS
RED CELLS
LOWER ANTERIOR GINGIVAE
YELLOW: Mean = 89.4 Range = 31-100
Standard Deviation = 12.2
Average Deviation = 7.8
Range Ratio = 77.2 = 100
RED: Mean = 10.3 Range = 0-69
Standard Deviation = 12.3
Average Deviation = 7.6
Range Ratio = 0-22.5

FIG. 4.

3. *Cheek* (Fig. 3)

Red staining cells:	Average number = 73 Standard deviation = 10.5 Range ratio = 62.5 - 83.5
Blue staining cells:	Average number = 27 Standard deviation = 10.5 Range ratio = 16.5 - 37.5

4. *Gingiva* (Fig. 4)

Yellow staining cells:	Average number = 89.4 Standard deviation = 12.2 Range ratio = 77.2 - 100
Red staining cells:	Average number = 10.3 Standard deviation = 12.3 Range ratio = 0 - 22.5

DISCUSSION

The above findings seem to indicate that there exists a pattern of cornification which appears to be constant for the various sites of the oral mucosa studied, and that this may be demonstrated by the use of the direct smear technic. This pattern confirms previous histologic findings.[8] It indicates complete cornification of the surface epithelium of the gingivae (yellow staining cells) with lesser degrees of cornification on the dorsum of the tongue, followed by the cheek and the ventral surface of the tongue.

Regardless of the degree of cornification, there is a continuous shedding of cells, all of which belong to the surface layer of the epithelium. In none of the normal cases studied were any cells of the basal layer found.

The apparent constancy of this cornification pattern would seem to indicate that a normal base line might exist which may be utilized in studies of abnormal conditions. It is of interest to note that this technic may also be used as an adjunct in the diagnosis of oral malignancies and as a means of following the results of therapy.

SUMMARY AND CONCLUSIONS

1. Four sites of the oral mucosa were selected for a study of the degree of cellular cornification, employing the direct smear technic of Papanicolaou.

2. Fifty men students of New York University College of Dentistry, ranging in age from 20 to 30 years, were used for this investigation.

3. The findings were as follows*: (a) dorsal surface of the tongue: 86 per cent red staining cells, 14 per cent blue staining cells; (b) ventral surface of the tongue: 21 per cent red staining cells, 79 per cent blue staining cells; (c) cheek: 73 per cent red staining cells, 27 per cent blue staining cells; and (d) gingivae: 89.4 per cent yellow staining cells, 10.3 per cent red staining cells.

*The classification of the types of cells and their relation to cornification follows that outlined by Papanicolaou.

4. The results indicate the degree of cornification to be highest at the gingivae followed by the dorsum of the tongue, the cheek, and the ventral surface of the tongue in the order named.

5. These findings are in accord with previous histologic evidence.

Grateful acknowledgment is made to Drs. Louis A. Wolfe, Sidney Sorrin, and Louis R. Burman for their helpful suggestions and their aid in the preparation of this paper.

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A STUDY OF EXFOLIATIVE CYTOLOGY OF NORMAL HUMAN ORAL MUCOSA

PAUL W. MONTGOMERY, D.D.S., M.S.

From the Departments of Pathology and Dentistry, Ohio State University, Columbus, Ohio

INTRODUCTION

INTEREST in the exfoliative cytology of the oral cavity dates back as far as the end of the ninteenth century when Miller[1] in 1890 described epithelial cells and leucocytes in human saliva. Probably the first extensive study of this subject was made by Orban and Weinmann,[2] in 1939; they investigated the cellular contents of the saliva in patients with dental caries. In 1940, Weinmann[4] studied the process of keratinization of the oral mucosa with various cytologic methods. He selected the Ernst-Gram and Wright methods as the most suitable stain and demonstrated the various stages of cornification of oral epithelial cells. He also studied the distribution of these cell types in the various regions of the mouth, but did not extend the investigation to pathologic conditions. Ziskin Kamen and Kittay[5] (1941) investigated the normal process of keratinization and keratinization in such conditions as lupus erythematosus, lichen planus, and leucoplakia by a similar method. The patterns of keratinization indicated by the smear method were similar to those previously observed by biopsy in both normal and abnormal tissues. A later study[6] (1942), which compared oral and vaginal smears from patients with certain gynecologic disorders, indicated a certain degree of parallelism in the process of keratinization between oral and vaginal mucosa.

The method used in this investigation is that of Papanicolaou and Traut[3] who for many years have studied epithelial smears in human beings and laboratory animals. Papanicolaou's early studies included an extensive investigation of the normal cellular patterns in epithelial smears prepared from vaginal secretion. His work subsequently involved the cytologic investigations of pathologic conditions and by 1928 he was able to demonstrate cancer cells in vaginal smears successfully. By 1940 his technic was sufficiently perfected to be put into pratical use in the gynecology clinic of the New York Hospital, where it has been in constant use since. With the increasing popularity of this method as an aid in diagnosing cervical and uterine cancer, the question of its value as an aid in diagnosing malignancies occurring at other sites has arisen. Fluid from the pleural and peritoneal cavities, sputum, urine, and gastric washings have been studied. Because of the proved usefulness of this method, it was felt that its application to lesions of the oral cavity might be successful.

This investigation was supported in part by a teaching aid grant from the National Cancer Institute of the Public Health Service.

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However, before the method could be effectively evaluated as an aid in diagnosing lesions of the oral cavity, it seemed necessary to use the technic in a study of normal exfoliative cytology of the mouth. For this reason the present investigation was undertaken.

MATERIAL AND METHODS

In order to evaluate the exfoliative cytology of the normal human oral mucosa, 75 individuals with clinically normal mouths were studied. This group was composed of 25 children between the ages of 3 and 10 years, 25 adults between the ages of 20 and 40, and 25 elderly individuals, above 63 years of age. Smears were prepared from material collected from the following six regions of each mouth: an area in the soft palate on its lateral and posterior aspect; an area in the cheek opposite the occlusal plane in the first molar region; an area in the labial vestibule in the lower lateral incisor region; an area on the dorsal surface of the tongue near the tip and lateral to the midline; an area on the dorsum of the tongue in its middle third and lateral to the midline; and an area on the gingiva in the lower anterior region between the central and lateral incisor.

Since it has been suspected that the oral mucosa of women might undergo cyclic change similar to that of the vaginal mucosa, smears from the oral mucosa of women were examined during various stages of the menstrual cycle. Two young women were chosen and smears made from the six areas of the oral mucosa at weekly intervals for a 4-week period. This was repeated for a second period of time by examining one individual over a 5-week period and the other over a 6-week period. The experiment was controlled by smears taken from 3 young men of a similar age group at similar intervals.

To collect the material the mucosa was gently scraped with a Woodson's No. 2 metal plastic instrument. As this instrument has a relatively dull edge, it merely collected the surface cells, without hemorrhage or other injury to the tissue. The material collected in this manner was spread on a clean glass slide and fixed immediately in a solution of equal parts of 95 per cent ethyl alcohol and ethyl ether. All material was fixed for a minimum of 20 minutes. The staining method used here was the method outlined in the monograph of Papanicolaou and Traut.[3]

To test the effect of the pH of the environment upon the staining method, a solution of acetic acid buffered to a pH of 4.5 and a solution of saturated sodium bicarbonate of approximate pH 8.5 were used. Three individuals with clinically normal mouths were selected and run through a series of three sets of smears at weekly intervals over a 3-week period. The first week the acid mouth wash was used, the second week the alkaline solution, and the third week plain tap water. The individual was asked to rinse his mouth for 2 minutes with the solution immediately before smears were prepared from each of the usual sites on the oral mucosa. The material was fixed and stained by the usual method. No significant differences in the staining results could be noted.

Smears stained by this method were then studied under the microscope, using magnifications of ×120 and ×450. With some practice, various cell types could be distinguished, the characteristics of which are given in Table I.

Since the small blue cells, the large mixed cells, and the red-yellow cells were relatively rare, it seemed most practical to simplify this classification and classify all these cell types into three major groups: the blue cells, the red cells, and the yellow cells. Therefore, the small blue cells were included in the blue groups, and the large mixed cells and red-yellow cells were placed according to the color predominating in their cytoplasm. These cell types represent the various stages of keratinization of the squamous epithelial cell, ranging from the nonkeratinized blue cell to the highly keratinized yellow cells.

In addition to the varying staining qualities of the cytoplasm, several other differences were noted in these epithelial cells. They varied greatly in size, the small blue and the yellow cells usually being the smallest and the large blue and large mixed cells usually being the largest. They appeared in various shapes, ranging from almost round types to elongated forms. In some cells the cytoplasm was quite clear, while that of others was granular or showed particulate matter in the cytoplasm. The nuclei, too, varied in size and staining qualities. The largest nuclei were seen in the small blue cells. In the other types, the size of the nucleus seemed to vary according to the size of the cell, the larger cells possessing the larger nuclei. The nuclei were round or oval in shape, and stained deeply with hematoxylin. Usually the small nuclei stained more darkly. Some nuclei presented one or two nucleoli and many of them showed clumping of the chromatin. In the red-yellow cells the nucleus was small and stained less intensely, and in the yellow type it appeared very faint or was absent entirely.

TABLE I

CHARACTERISTICS OF EPITHELIAL CELLS OF THE ORAL MUCOSA

DENOMINATION	NUCLEUS (SIZE)	CYTOPLASM (AMOUNT)	CYTOPLASM (COLOR)
Small blue	Large	Small	Blue
Large blue	Small	Large	Blue
Large mixed	Small	Large	Blue-red
Large red	Small	Large	Red
Red-yellow	Small	Small	Red-yellow
Yellow	Absent	Small	Yellow

Certain other cells, such as leucocytes and histiocytes, were noted. Various microorganisms that normally inhabit the mouth and varying amounts of debris were present.

In order to show the distribution of the three main types of epithelial cells in each smear, differential counts were made. This consisted of counting the first hundred cells encountered which occurred singly or in small clumps. Large clumps of cells were not included. These figures were grouped in table form according to each individual and the various regions of the mouth studied. These data were then submitted to the Ohio State University's Statistics Laboratory for analysis.

These data were tested as being grouped according to the color of the cells in a certain part of the mouth in a certain age group. The mean of the items in each group can be considered as a random variable. The material was treated by calculating the confidence interval. A confidence interval is an interval computed in such a manner that it would contain the true mean of a group of items a certain fixed per cent of the time (in this instance, 95 per cent).

Computations were made according to the formula:

$$\text{C interval} = \frac{\sum_{k=1}^{r} n_k p_k}{\sum_{k=1}^{r} n_k} \pm t_{.05} \sqrt{\frac{\sum_{k=1}^{r} n_k p_k - \frac{\left(\sum_{k=1}^{r} n_k p_k\right)^2}{\sum_{k=1}^{r} n_k}}{\sum_{k=1}^{r} n_k \; r}}$$

In this formula, k denotes the number of the person, n_k the number of cells counted for the k^{th} person, and p_k the percentage of a certain colored cell for the k^{th} person, while r denotes the number of persons in the group and $t_{.05}$ is taken from a table of the "t" distribution for r-1 degrees of freedom.

If the confidence intervals of two particular groups do not overlap, the means of these groups are significantly different on the 5 per cent level.

RESULTS

Space does not permit the tabulation of all individual differential counts made in this study. A graphic demonstration of the average percentage distribution of the three cell types in the various oral regions in the three age groups is illustrated in Fig. 1.

Our results indicated no particular relationship between age and sex and the distribution of cell types in different parts of the mouth. Consequently these data of the three age groups were combined in Table II.

TABLE II

PERCENTAGE DISTRIBUTION OF CELLS

REGION	CELL TYPE	MEAN PERCENTAGE	STANDARD DEVIATION	CONFIDENCE INTERVALS
Soft palate	Blue	50.8	28.8	35.96-77.69
	Red	41.7	27.2	19.82-54.70
	Yellow	7.5	12.9	0.31-20.81
Cheek	Blue	51.5	26.6	37.81-62.48
	Red	43.8	23.8	25.89-60.29
	Yellow	4.7	12.3	0.0 - 9.54
Vestibule	Blue	45.0	28.9	25.29-64.74
	Red	42.9	26.8	28.77-64.01
	Yellow	12.1	21.7	0.50-30.11
Tongue (ant.)	Blue	19.7	16.7	6.93-31.74
	Red	47.3	20.7	33.01-58.33
	Yellow	33.7	21.4	19.32-47.24
Tongue (post.)	Blue	30.9	16.8	17.77-45.34
	Red	35.5	15.9	25.20-47.19
	Yellow	33.5	15.4	23.27-42.09
Gingiva	Blue	7.3	9.1	0.0 -13.41
	Red	17.8	18.1	4.51-33.30
	Yellow	75.0	21.5	56.98-83.65

From Table II it can be seen that the soft palate and the cheek show patterns which are almost identical with a predominance of blue cells, a slightly smaller number of red cells, and very few yellow cells. The vestibule area shows a similar pattern, except for a slight increase in the number of yellow cells. The two tongue regions show a predominance of the red cells and in both regions approximately one-third of the cells were of the yellow type. The posterior

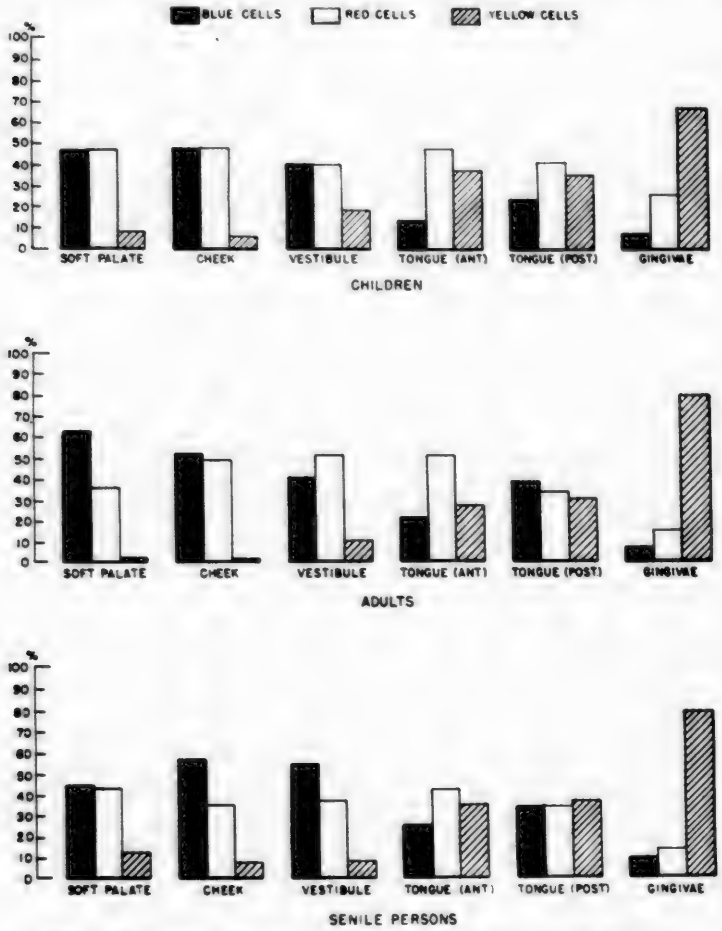

Fig. 1.—Percentage distribution of cell types in normal oral mucosa.

tongue region showed a greater proportion of blue cells than the anterior tongue region. The gingiva differs from all the other sites studied because of its high incidence of yellow cells, with red cells next in prominence, and blue cells less commonly encountered.

Statistical analysis on the basis of overlapping confidence interval values shows valid differences existing between: blue cells of the palate, cheek, and

vestibule areas and those of the two tongue regions and the gingivae; red cells of the gingivae and all five other regions; yellow cells of the gingivae and all five other regions.

In some cases, a marked deviation from the mean percentage distribution was encountered. For example, one 65-year-old man and one 10-year-old child showed 82 and 95 per cent yellow cells, respectively, in smears from the cheek, which has a mean of 4.7 per cent. On the other hand, one 8-year-old child and one 70-year-old man showed only 2 and 12 per cent yellow cells in the smears from the gingivae, which has a mean of 75 per cent. At present, no satisfactory explanation for these marked deviations from the normal percentage distribution can be given but it will be interesting to follow these two individuals for possible pathologic changes.

Finally, the possibility of any changes which might occur in the oral mucosa during the menstrual cycle was investigated. Counts on smears taken at weekly intervals on 2 women and 3 men were studied. The statistical analysis of these data was based upon the departure from randomness as indicated by the number of variations. The values were found to be not significantly different from what one would expect to actually occur at random. There was no evidence of any periodic fluctuation in oral epithelial cells during the estral cycle.

The study of a smear from the oral cavity would not be complete without paying attention to the other details found in the smears aside from the epithelial cells. Probably the most constant finding was the many types of oral bacteria in varying numbers. They could be found in most smears, some showing few and others great numbers. The bacteria were either within the cytoplasm of the cell or were closely adherent to the surface of the cell. In some slides they occurred as large clumps not associated with the cells. Some smears showed also a varying number of fungi. These occurred most frequently in smears from the posterior portion of the tongue and from the gingiva.

Leucocytes were present in some of the smears in varying numbers. These appeared to be chiefly polymorphonuclear neutrophils and were in varying stages of degeneration and disintegration. Histiocytes were rarely found and no erythrocytes were noted. A varying amount of mucous material and debris was always present. The debris was found chiefly in smears prepared from the tongue and gingival areas.

There was no correlation between the bacterial flora, the number of leucocytes, or the amount of debris with the cytologic picture of the smears.

SUMMARY AND CONCLUSIONS

1. The normal cytologic picture of the oral cavity was studied by means of cell smears stained with Papanicolaou's method in 80 normal individuals of varying age and sex.

2. Differential counts of the three types of epithelial cells found showed definite cytologic patterns in various regions of the mouth which were proven statistically significant.

3. Rather drastic deviations from the normal cytologic patterns were observed occasionally without explanation for their occurrence.

4. Age, sex, or the menstrual cycle does not significantly influence the cellular pattern of the oral mucosa as explored by this method.

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GINGIVAL CHANGES IN DEFICIENCY STATES, PREGNANCY, AND LACTATION PERIOD

VIMLA SUD, B.D.S. (Pb)*

Eastman Dental Dispensary, Rochester, N. Y.

IT has been generally accepted that gingivitis and other periodontal conditions are often the oral manifestations of a variety of general and systemic factors and diseases. This predisposition to disease is believed to be the result of nutritional metabolic or endocrine disorders and may be activated by local factors such as infection, calcareous deposits, faulty oral hygiene, etc.

An opportunity to study the relationship between some of these factors arose when a large number of sorely distressed refugees from the West Punjab were concentrated in better organized refugee camps like the Gandhi Nagar Camp at Jullundur in the East Punjab. In this camp, which housed about 20,000 men, women and children, full cooperation was available to the staffs responsible for the nutritional survey, the antenatal clinic, and the maternity section of the camp hospital.

Since the situation presented unique research material, the opportunity was utilized to investigate the role which nutritional deficiencies, pregnancy, and lactation played in periodontal disease.

Because previous studies[1, 2, 3] have drawn attention to marginal and hypertrophic gingivitis as early manifestations of periodontal disease, it seemed important to devote attention to conditions of the gingiva which could serve as an index of changes brought about by the general factors of vitamin deficiency, pregnancy, or lactation.

MATERIAL AND METHODS

For the purpose of this study, 248 individuals were examined. These included 148 women and 100 men, who were further subdivided as shown in Table I. Thirty-five of the women and 14 of the men were younger than 20 years of age. Thirty-three of the men and 12 of the women were older than 40 years of age.

TABLE I

SUBJECTS	MEN	WOMEN		
		NONPREGNANT	PREGNANT	POSTNATAL
Showing frank features of vitamin deficiency	50	25	25	25
Not showing frank features of vitamin deficiency	50	25	23	25
Total	100	50	48	50

No selection was exercised and subjects were picked at random from among the refugee population. The material was, therefore, fully representative and comprised a group of an equivalent number of men with and without frank signs of vitamin deficiency, and an equivalent number of women (nonpregnant

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*Present address: Jullundur, East Punjab, India.

and at various stages of pregnancy) with or without frank signs of deficiency, and also females in the postnatal phase.

It should also be understood that even subjects who showed no definite or specific vitamin deficiency were malnourished. Due to camp conditions, biochemical investigations were not possible, but clinical criteria used by the nutrition research workers sufficed to classify the patients according to the most apparent vitamin deficiency.

The oral examination procedure entailed a careful study of the mouth as a whole with mirror and explorer and the use of calibrated blunt probe for estimation of pocket depth. The examination was conducted in good natural light in open air.

TABLE II

GINGIVITIS

SUBJECTS		DEGREE I*		DEGREE II		DEGREE III		AVERAGE DISEASE† FIGURE	
TYPE	NUMBER	UPPER	LOWER	UPPER	LOWER	UPPER	LOWER	UPPER	LOWER
		%	%	%	%	%	%		
Males									
Deficient	50	32.0	32.0	40.00	40.0	28.0	28.0	1.96	1.96
Nondeficient	50	42.0	22.0	44.00	68.0	14.0	10.0	1.72	1.88
Females									
Deficient nonpregnant	25	40.0	28.0	36.00	52.0	24.0	20.0	1.82	1.92
Deficient pregnant	25	32.0	12.0	48.00	60.0	20.0	28.0	1.88	2.16
Deficient postnatal	25	40.0	20.0	44.00	60.0	16.0	20.0	1.76	2.00
Nondeficient nonpregnant	25	40.0	28.0	40.00	60.0	20.0	12.0	1.8	1.84
Nondeficient pregnant	23	69.5	30.4	2.17	60.8	8.6	8.6	1.39	1.78
Nondeficient postnatal	25	64.0	36.0	24.00	44.0	12.0	20.0	1.48	1.84

*If 2 gingivae out of 8 are affected, it is Degree I.
If 4 gingivae out of 8 are affected, it is Degree II.
If more than 4 gingivae are affected, it is Degree III.

†Average disease figure is addition of Degree I one time: Degree II two times: Degree III three times, divided by total number:

$$\frac{\text{I-II-II-III-III-III}}{\text{total number}} = \text{ADF}$$

The criteria followed for the assessment of the gingival condition were those suggested by King[4] and used by Day and Shourie.[1] Eight gingival regions were examined between the canines and second incisors and between the second and first incisors of both upper and lower jaws. However, due to the natural brown to black pigmentation in most of the individuals, it was difficult to assess normality in terms of color, or to evaluate the severity of disease from the degree of redness of color. Sometimes natural pigmentation also reduced the diagnostic value of digital pressure and recovery tests.

The gingiva were carefully examined for color changes, swelling of the margin and papillae (hypertrophic gingivitis), ulceration, pocket formation, hemorrhage and suppuration, and the teeth for calculus and debris. Cleansing habits were also studied.

FINDINGS

Variation in Color of Facial and Oral Tissues.—No relation between facial complexion and the color of oral mucosa could be observed.

TABLE III

GINGIVAL CONGESTION

SUBJECTS		DEGREE I	DEGREE II	DEGREE III	TOTAL COLOR CHANGE	AVERAGE DISEASE FIGURE
TYPE	NUMBER	UPPER & LOWER	UPPER & LOWER	UPPER & LOWER	UPPER & LOWER %	UPPER & LOWER
Males						
Deficient	50	9	11	23	86.0	2.00
Nondeficient	50	6	11	21	76.0	1.82
Females						
Deficient nonpregnant	25	6	4	14	96.0	2.24
Deficient pregnant	25	3	6	15	96.0	2.4
Deficient postnatal	25	2	6	14	88.0	2.24
Nondeficient nonpregnant	25	2	6	10	72.0	1.76
Nondeficient pregnant	23	5	1	13	82.6	2.00
Nondeficient postnatal	25	2	10	8	80.0	1.85

Gingival Hypertrophy.—From Table II it can be seen that all patients showed slight to severe degrees of proliferative hypertrophic gingivitis. In the lower jaw this was more marked in extent and degree than the upper jaw. A comparison of the average disease figure for subjects of various groups showed a universally high figure in all of the nutritionally deficient men and women.

TABLE IV

GINGIVAL ULCERATION

SUBJECTS		DEGREE I	DEGREE II	DEGREE III	TOTAL IN ALL	AVERAGE DISEASE FIGURE
TYPE	NUMBER	UPPER & LOWER	UPPER & LOWER	UPPER & LOWER	UPPER & LOWER (%)	UPPER & LOWER
Males						
Deficient	50	3	6	9	36.0	0.84
Nondeficient	50	1	4	8	26.0	0.66
Females						
Deficient nonpregnant	25	4	1	4	36.0	0.72
Deficient pregnant	25	2	5	5	48.0	1.08
Deficient postnatal	25	1	4	–	20.0	0.36
Non-deficient nonpregnant	25	2	2	4	32.0	0.72
Nondeficient pregnant	23	3	2	5	43.4	0.95
Nondeficient postnatal	25	–	–	2	8.0	0.24

TABLE V

GINGIVAL HEMORRHAGE

SUBJECTS		DEGREE I		DEGREE II		DEGREE III		AFFECTED		AVERAGE DISEASE FIGURE	
TYPE	NUMBER	UPPER	LOWER	UPPER	LOWER	UPPER	LOWER	UPPER (%)	LOWER (%)	UPPER	LOWER
Males											
Deficient	50	13	12	5	7	2	7	40.0	52.0	0.58	0.94
Nondeficient	50	11	11	4	7	1	3	32.0	42.0	0.44	0.68
Females											
Deficient nonpregnant	25	3	5	5	3	1	3	36.0	44.0	0.64	0.8
Deficient pregnant	25	2	7	1	4	3	3	24.0	56.0	0.52	0.96
Deficient postnatal	25	6	7	8	8	1	3	60.0	72.0	1.0	1.28
Nondeficient nonpregnant	25	3	3	1	2	3	4	28.0	36.0	0.56	0.76
Nondeficient pregnant	23	3	3	-	1	2	4	21.7	34.7	0.39	0.73
Nondeficient postnatal	25	2	5	2	1	2	3	24.0	36.0	0.48	0.64

The relationship between malnutrition and gingivitis seems evident. The highest average disease figure (2.16)[4] was found in the lower gingiva of women showing pregnancy as well as deficiency. Third degree gingivitis was found in the lower jaws of 28 per cent of this group.

Gingival Congestion.—Among the men, 86 per cent of the deficient and 76 per cent of the nondeficient groups presented a maroon congestive color (Table III). Among the women, 96 per cent of the deficient group showed congestion, except the deficient postnatals, in which 88 per cent had gingival congestion. In the women who were not deficient, only 72 per cent (nonpregnant), 80 per cent (postnatal), and 82.6 (pregnant) had congestion. The color changes in the pregnancy group were similar to those described by Ziskin.[5]

Gingival Ulceration.—Frequently the gingiva showed chronically ulcerated areas (Table IV). The percentage of subjects with ulceration was uniformly higher in the deficient group than in the nondeficient group: 36 per cent as compared with 26 per cent in the men; 36 per cent compared to 32 per cent in nonpregnant women; 48 per cent compared to 43.4 per cent in pregnant women; and 20 per cent compared to 8 per cent in postnatal women. Another interesting finding is that 34 per cent of the nonpregnant women showed ulceration with an average disease figure (ADF) of 0.72, whereas in pregnant women this occurred in 45.7 per cent with an ADF as high as 1.02. That pregnancy plays a part in the causation of gingival ulceration seems clear.

Gingival Hemorrhage.—The degree of hemorrhage resulting from firm digital pressure on the gingiva also was observed (Table V). The lower gingiva showed a greater tendency to hemorrhage than the upper. A larger percentage of deficient individuals had hemorrhage and the group had a higher average hemorrhage figure. The deficient pregnant women showed a higher percentage and a higher average hemorrhage figure than the nondeficient pregnant women. It will also be observed that the grossest form of hemorrhage (an average hemorrhage figure of 1 and 1.28 in upper and lower gingiva, respectively) was most common in the deficient postnatal women. This suggests that, probably due to continued avitaminosis, the hemorrhaging tendency set up during pregnancy continued during the postnatal phase.

Gingival Pockets.—Periodontal pockets of varying depths were present in almost all patients. Measurements from the base of gingival crevice to the free margin of the gingiva were made in labial, lingual, and interdental regions in both upper and lower jaws.

Table VI summarizes the results. It will be seen that the interdental pockets were deeper than the labial and lingual pockets in all groups of subjects. No significant differences in pocket depths could be made between the various groups.

Suppuration.—The presence and extent of suppuration in the pockets was also observed, and the percentages of each group showing suppuration were as follows: In the deficient and nondeficient groups, respectively: men, 72 per cent and 44 per cent; nonpregnant women, 40 per cent and 24 per cent; pregnant women, 36 per cent and 30 per cent; and postnatal women, 40 per cent and

Table VI

Depth of Periodontal Pockets

Subjects			Depth labial pockets			Depth lingual pockets			Depth interdental pockets			
Type	Number		Up to 1 mm.	Up to 2 mm.	Up to 3 mm.	Up to 1 mm.	Up to 2 mm.	Up to 3 mm.	Up to 1 mm.	Up to 2 mm.	Up to 3 mm.	Up to 4 mm.
Males												
Deficient	50	Affected subjects	14	32.00	4.00	33	17.00	-	4	20.00	21.00	5.00
		Average depth	1	1.60	2.10	1	1.70	-	1	1.56	2.49	3.38
Nondeficient	50	Affected subjects	12	34.00	4.00	32	12.00	-	1	21.00	26.00	2.00
		Average depth	1	1.41	2.17	1	1.60	-	1	1.86	2.45	3.40
Females												
Deficient nonpregnant	25	Affected subjects	8	16.00	1.00	18	7.00	-	1	15.00	9.00	-
		Average depth	1	1.48	2.30	1	1.60	-	1	1.76	2.32	-
Nondeficient nonpregnant	25	Affected subjects	9	14.00	2.00	20	5.00	-	-	16.00	8.00	1.00
		Average depth	1	1.52	2.25	1	1.64	-	-	1.90	2.30	3.10
Deficient pregnant	25	Affected subjects	5	20.00	--	19	6.00	-	-	11.00	12.00	2.00
		Average depth	1	1.49	--	1	1.48	-	-	1.92	2.51	3.75
Nondeficient pregnant	23	Affected subjects	10	12.00	1.00	15	8.00	-	-	12.00	9.00	2.00
		Average depth	1	1.32	2.30	1	1.27	-	-	1.90	2.30	3.10
Postnatal deficient	25	Affected subjects	6	17.00	2.00	20	4.00	1	1	11.00	12.00	1.00
		Average depth	1	1.42	2.60	1	1.62	3	1	1.90	2.44	4.00
Postnatal nondeficient	25	Affected subjects	11	10.00	4.00	17	8.00	-	-	12.00	10.00	3.00
		Average depth	1	1.54	2.30	1	1.57	-	-	1.85	2.31	3.46

28 per cent. The high percentage figure for the deficient men is the most interesting finding in this series of observations.

Gingival Changes in Relation to Period of Pregnancy.—An effort was also made to compare the gingival changes at various months of pregnancy. In 13 women pregnant for less than 6 months, 11 showed change of gingival color of which 6 were first degree, 4 second degree, and 1 (6 months) third degree. Of the 32 women with changes and more than 6 months pregnant, 4 had first-degree, 5 second-degree, and 23 showed third-degree change. This indicates strongly that the extent of congestion advances with the stage of pregnancy. Parallel observations were noted in gingival ulceration, hemorrhage, and degree of gingivitis.

Gingival Changes in Relation to Various Vitamins.—The number of cases of gingival pathosis, demonstrated in the various groups of vitamin deficiencies, was as follows:

TYPE OF VITAMIN DEFICIENCY	NUMBER
A	12
B	3
B_2	54
B complex	11
A, B_2, and C	4
A and B complex	17
A and B_2	16
B_2 and C	5

The complexity of this grouping and the relatively small number of cases make definite conclusions impossible. The predominant deficiency was in the vitamin B complex. No cases of pure vitamin C and D deficiency were seen. The gingival changes appeared to be almost equally distributed among all vitamin deficiency groups. This suggests that they were the result of a state of general avitaminosis.

Incidence of Supragingival, Subgingival, and Mixed Calculus.—The percentages of surfaces upon which various amounts and types of calculus occurred were noted for all patients and are recorded in Table VII. Gingivitis was present in all surfaces of all gingiva examined in all groups but the percentages of surfaces showing calculus varied from 48.7 per cent to 73 per cent. It appears that gingivitis occurred independently of calculus, but that it was more severe when calculus was present.

Subgingival calculus was the most common of the types differentiated. It was also associated with a higher average gingival disease figure. The patient groups with the higher incidence of gingivitis (deficient groups) showed higher incidence of calculus and higher disease figures. This suggests that calculus had aggravated the gingivitis and not initiated it. That the calculus was not the principal cause of the gingivitis is suggested by the presence of gingivitis where no calculus was present.

Debris and Plaques.—While a large percentage of patients showed both plaques and debris, no relationships could be made out between subjects or groups.

Cleansing Habits.—Most subjects did not pay much attention to oral hygiene. None used a toothbrush. They used the twig (Dattan) as is the

Indian custom, but cleaned their teeth only once or twice a week. Nearly similar conditions prevailed in all groups. Since the lack of oral hygiene was equally marked in all groups, it could not be compared with gingival disease.

TABLE VII

OCCURRENCE OF CALCULUS

SUBJECTS		TOTAL NO. OF SURFACES OF TEETH EXAMINED	AFFECTED SURFACES (%)	SUPRA-GINGIVAL CALCULUS (%)	SUB-GINGIVAL CALCULUS (%)	MIXED CALCULUS (%)
TYPE	NUMBER					
Males						
Deficient	50	710	73.1	22.6	36.6	13.8
Nondeficient	50	794	62.2	25.3	27.7	9.1
Females						
Deficient nonpregnant	25	386	56.9	18.2	26.1	12.4
Nondeficient nonpregnant	25	386	51.2	28.7	14.2	8.2
Deficient pregnant	25	400	66.5	26.2	30.2	10.0
Nondeficient pregnant	23	368	50.2	19.5	19.8	10.8
Postnatal deficient	25	392	55.8	28.8	22.7	4.3
Postnatal nondeficient	25	396	48.7	21.2	18.9	8.5

COMMENT

That the periodontal conditions observed were the result of multiple deficiencies is supported by the facts that the author was able to find manifest signs of specific vitamin deficiencies in only 6.3 per cent of the patients examined, and that the nutritional workers,[6] who studied the patients, concluded that pathosis of the gingival tissues was attributable to states of general malnutrition rather than specific vitamins. This is in accord with the opinion of Spies, Bean, Wilter, and Huff[7] who, after a study of riboflavinosis, laid stress on the importance of multiple vitamin deficiencies in the causation of tissue disease.

The findings have revealed a relationship between advancing months of pregnancy or the phase of lactation and gingival disease. It is felt this may be the effect of either relative malnutrition or hormonal influences. Malnutrition would be the result of pregnancy throwing a further demand on maternal organism. The observation that the severest forms of gingivitis were present in malnourished pregnant women supports this hypothesis. The possible effect of hormonal influences is suggested by the relationship between "general hyperplasia of the gums" and the activity of anterior pituitary lobe (Perkoff[8]) in which imbalance occurs during pregnancy. This point, however, requires further investigation.

In any investigation of disease, it is extremely difficult to evaluate the relative importance of systemic and local influence which contribute to its causation. In this study the difficulty was heightened by unusual conditions which bore upon the refugee population. In addition to physical exhaustion and anxiety, many of the patients had suffered from infectious disease and malaria, which

had undermined their health and resistance, thus exaggerating any systemic predisposition, nutritional or otherwise, which might contribute toward periodontal disease. There were also special local factors which would tend to affect the gingiva adversely. One of these was that in many of the subjects the long travel under hurried and dangerous circumstances had established a habit of mouth breathing which might affect the gingiva adversely. Under such circumstances it was inevitable that oral hygiene was neglected, thereby adding further to the local irritation of the gingiva. Because of the food shortage and hunger suffered by the refugees in transit, they apparently devoured anything edible they found, without regard to its harmful effect on the teeth and gingiva. There was much evidence of traumatic injury in their mouths, which undoubtedly served as a starting point for infection. However, because these factors applied equally to all groups of refugees studied, the differences between the groups seem to provide a reasonable basis for tentative conclusions. Therefore, because the most marked gingival pathosis and suppuration were found in those exhibiting deficiencies, particularly the pregnant women, it seems justifiable to suggest that nutritional deficiency played a predominant part in etiology of the gingival disease observed in this series of cases.

SUMMARY

A comparison has been made of the gingival conditions of 248 Indian refugees who were grouped according to various stages of nutritional deficiency and including patients in pregnancy and postnatal states.

Gross changes in the gingivae, including congestion, ulceration, hypertrophic gingivitis, and an increased tendency to hemorrhage, were most marked in the pregnant groups.

Similar changes were also associated with malnourishment, and the worst form of gingival pathosis was found in women who were suffering from nutritional deficiencies during pregnancy.

The existence of local factors such as calculus accentuated the effects of nutritional deficiencies on the development of gingivitis.

The author wishes to thank the Medical Commissioner Refugees, East Punjab, for having kindly permitted her to carry out this investigation. She wishes to acknowledge gratefully the cooperation extended by Drs. Berrington and Singh of the Nutritional Survey of the camp, for making examinations of all cases, to express indebtedness to Dr. Nirmala Devi Chand of the Maternity Section of the Camp Hospital for granting permission and helping in the examination of postnatal cases, and to acknowledge the valuable help given by Dr. Basil G. Bibby, Director of the Eastman Dental Dispensary, in the preparation of this manuscript.

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HISTOCHEMICAL STUDY ON ALKALINE PHOSPHATASE IN NORMAL GINGIVAE, VARYING THE pH AND THE SUBSTRATE

RÓMULO LUIS CABRINI AND FERMÍN ALBERTO CARRANZA, JR.

Buenos Aires, Argentina

WHILE histochemical studies of alkaline phosphatase have been performed in nearly every tissue of the human body, including the tooth bud, there are rather few reports referring to this ferment in the gingiva. Zander[1] found enzymatic activity only in the endothelium of some vessels and sometimes in the collagenous fibers of the gingiva. Valloton[2] found phosphatase in the endothelium of some vessels and constantly in the gingival collagenous fibers. Since those reports, there have been no substantial changes of technic, except the systematic employment of different pH's and substrates.

MATERIAL AND TECHNIC

Clinically and histologically normal human gingivae were employed. The only pathologic change which was found was a slight lymphocytic infiltration in the corium of the epithelial attachment. Besides human gingivae, cat's and rabbit's gingivae and the skin of the plantar area of the cat were used for comparison.

Fixation was carried out with 80 per cent alcohol at low temperature (about 4° C.) during a varying span of time which did not last longer than 1 week.

Embedding was done in paraffin in the usual way and the phosphatases were demonstrated by the Gomori technic. Sodium glycerophosphate, nucleic acid, and lecithin were employed as substrates. The pH ranged from 6.4 to 9.2 for glycerophosphate, and with the other substrates it was 9.2. Each reaction was controlled by incubation without the substrate. Magnesium ion in form of 2 per cent magnesium chloride was employed as the catalyzing agent.

RESULTS

Sodium Glycerophosphate.—The enzymatic reaction is sharply defined and exclusively in the corium. It is more intensive in the region of the epithelial attachment (Fig. 3). The ferment is distributed preferentially around the vessels and in the collagenous fibers (Fig. 1). Obviously not all the vessels show the same reaction, and the reaction is not the same over the whole course of the same vessel. There are areas which remained practically free of enzymes.

The reaction is seen in the entire thickness of the capillaries (Fig. 1) while in the vessels of larger diameter the enzymatic reaction is condensed in the endothelium, being absent in the internal elastic membrana limitans and very faint in the muscular layer (Fig. 2). As pointed out the epithelium does not show any ferment except in the ends of papillae. (Fig. 1).

Below pH 7.2, the enzymatic activity weakens extraordinarily, requiring a week of incubation in order to become evident with 6.4 pH. Substantial variations in the distribution of the enzyme are not observed on changing the pH.

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Fig. 1.—Normal gingiva. Sodium glycerophosphate, incubated at pH 9.2 for 24 hours.

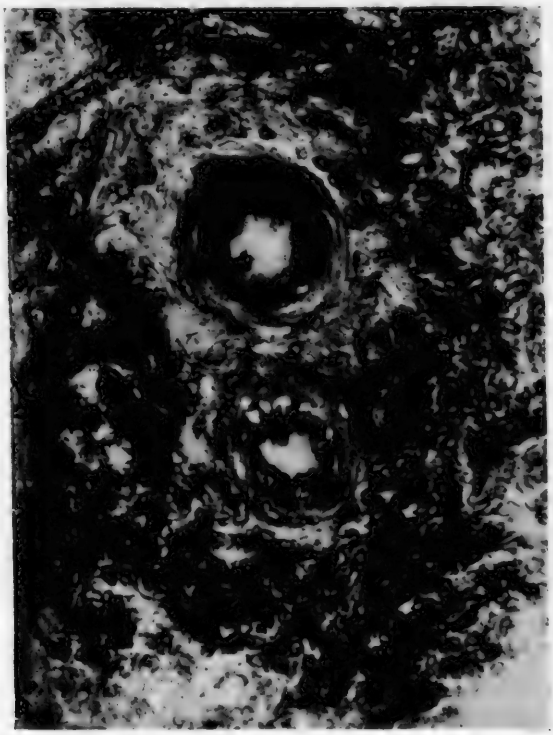
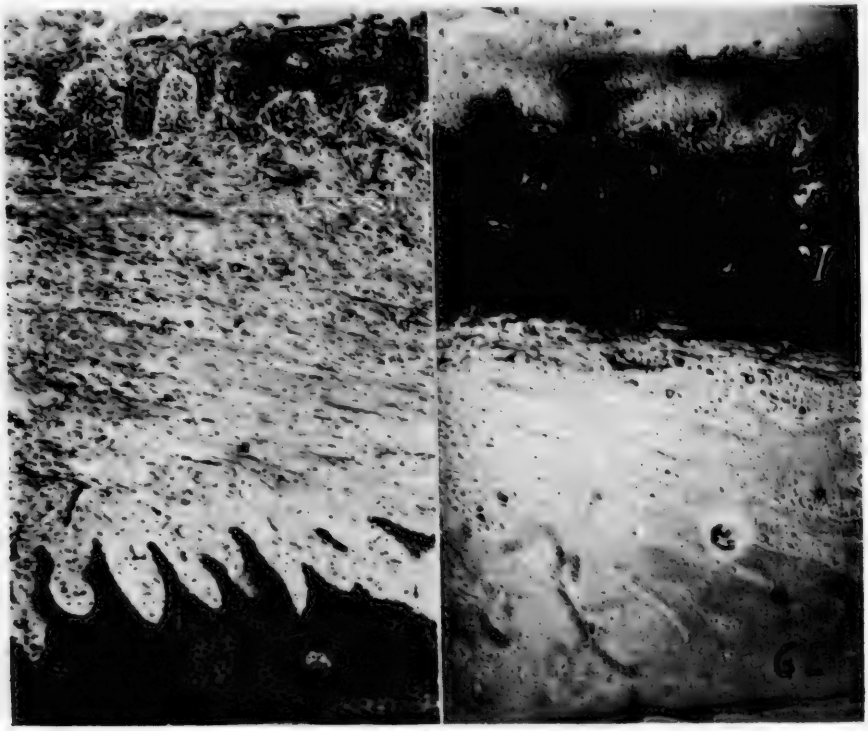


Fig. 2.—Two vessels in gingival corium. Sodium glycerophosphate, incubated at pH 9.2 for 24 hours.

Nucleic Acid.—The gingival phosphatase easily hydrolyzes the nucleic acid which appears as enzymatic reaction not only in the corium but also in the gingival epithelium (Fig. 4). In the corium the enzyme is distributed similarly to the hydrolysis of glycerophosphate. In the epithelium the ferment seems to condensate on the layer of the generative cells and in a lesser degree on the malpighian cells. In the higher strata there is almost no reaction.



A. *B.*

Fig. 3.—*EA*, Epithelial attachment. *GE*, Gingival epithelium. *I*, Infiltrate.
A, hematoxylin-eosin stain.
B, Sodium glycerophosphate, incubated at pH 9.2 for 24 hours.

Lecithin.—Hydrolysis of lecithin was attained only in the nuclei of the corium cells of the epithelial attachment, the enzymatic activity being almost negative in the epithelium, vessels, and collagen (Fig. 5).

DISCUSSION

The perivascular location of the phosphatase is a frequent occurrence in many organs including the adults dental pulp.[3] Nevertheless, the reason for such a location remains unkown.

In the vessels which are equipped with several layers (arteries and veins) most of the phosphatase activity is concentrated in the endothelium. Therefore, a certain relationship of the blood to the endothelium ought to be surmized. This would lead to two suppositions: one that the endothelium produces the phospha-

Fig. 4.—Normal gingiva. Nucleic acid, incubated at pH 9.2 for 72 hours.

Fig. 5.

Fig. 6.

Fig. 5.—Normal gingiva. Lecithin, incubated at pH 9.2 for 48 hours. *EA*, Epithelial attachment. *I*, Infiltrate cells.

Fig. 6.—Plantar skin of the cat. Sodium glycerophosphate, incubated at pH 9.2 for 24 hours. The apparent epithelial reaction is due to the presence of melanin. Heavy reaction in the sweat glands.

tase, releasing it into the blood, and the other that the phosphatase comes from the blood and is condensed in the endothelium in order to be carried to the tissues. A serious objection to the latter point of view is the entire lack of phosphatase in the internal elastic membrana limitans of the arteries. The most we can say is that, at least in the arteries, the phosphatase does not invade the surrounding tissue.

The gingival epithelium is free from hydrolyzing phosphatase of sodium glycerophosphate, a fact which was previously demonstrated by Zander[1] and Valloton.[2] A positive phosphatasic reaction has been found in the gingival epithelium by utilizing as a substrate the nucleic acid. However, this cannot be considered as a base for Adamson's theory on the formation of calculus through precipitation of calcium salt by action of the gingival phosphatase, as in that case the strongest condensation ought to be found in the surface layers.

The studies undertaken in other organs show clearly that phosphatase is present in the epithelium in a low degree except when the epithelium is either secretory or has an increased metabolic activity. A typical case in shown in Fig. 6 where only the secretory portion of the sudoriparous glands gives an intensive reaction. The slight activity found in hydrolysis of lecithin suggests that the phosphatase has certain difficulties to hydrolyze that substance in the gingiva as well as in other organs, as was shown by Dempsey and Deane.[4]

The ferment content found in the stratum granulosum of cat's skin has not been clear enough to draw conclusion; nevertheless, we can state, in disagreement with Smith and Parkhurst,[5] that we have never been able to observe a striking increase of enzymatic activity in these areas.

CONCLUSIONS

1. Enzymatic activity of phosphatase was observed especially in the corium adjacent to the epithelial attachment and is found condensed around the vessels and collagenous fibers.

2. In vessels with thick walls the strongest phosphatasic activity was found in the endothelium while the internal elastic membrana limitans showed a negative result.

3. The hydrolysis of glycerophosphate with different pH showed a definite decrease of phosphatasic activity as soon as the medium is rendered acid.

4. The hydrolysis of lecithin showed enzymatic activity in the nuclei of the corium of the epithelial attachment.

5. The hydrolysis of the nucleic acid showed the presence of the enzyme in the gingival epithelium, especially in the basal layers.

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ORAL MANIFESTATIONS OF IONIZING RADIATION

I. Oral Lesions and Effect on Developing Teeth of Swine Exposed to 2000 K.V. Total Body X-ray Irradiation

JAMES A. ENGLISH AND JOHN L. TULLIS

Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

INTRODUCTION

THIS is a report of an investigation of the oral tissues and developing teeth of 41 swine which were exposed to 2000 kv. total body x-ray irradiation. These swine were selected for study from a group of 120 animals that were being used in an experiment to determine the lethal dose of high voltage x-ray irradiation on large animals. In addition the experiment was to determine the effects of x-ray applied to one side of the body as compared to a bilateral exposure with the same dose of x-ray. Certain general pathologic responses will be published later; this publication is limited to a description of oral lesions and the effects of irradiation upon developing teeth, the tongue, and salivary glands.

The number of swine used in this study was not sufficient to provide good quantitative data. No statistical analyses have been attempted and the extent of quantitating has been resolved in simply stating how many animals responded in a certain way.

There are some interesting factors about developing teeth which make them especially valuable for radiobiologic study. They provide a means for the study of the effects of ionizing irradiation on cells of varied embryonal origin, in several stages of proliferation, growth, and differentiation. The enamel of teeth is produced by ameloblasts which differentiate from the inner enamel epithelium, which in turn is a product of the invagination of the oral epithelium. In the process of differentiation, these basal epithelial cells change their polarity[27] and become secretory in function. That is, the Golgi apparatus of the ameloblast moves to what was formerly the distal or outer end of the cell, and the enamel matrix is formed at the end of the cell which originally was its base (Fig. 1). This single cell type can, therefore, be studied in proliferation, differentiation (including polarity change), and productive metabolism. With these changes one naturally expects an alteration in its susceptibility to irradiation, for it has been shown that the same cell will respond to stimuli according to its metabolic activity, its state of growth, and its age.[6]

Other portions of the developing tooth also offer valuable material for similar comparative studies. The dentin of the tooth is formed by the activity of odontoblasts, which are differentiated from connective tissue contained in the dental papilla, and by the fibers of von Korff which occur among these connective tissue cells.[33] Here, then, is a connective tissue counterpart, in

The opinions or assertions contained herein are those of the writers and are not to be construed as being official or reflecting the views of the Department of the Navy or the naval service at large.

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close apposition to an epithelially derived structure, which can be studied throughout its stages of development. It should be pointed out that enamel and dentin (in differentiation to bone) serve as a permanent record of the activity of the blast cells which produce them. Failure of the blasts to produce matrix, or a faulty production, is irreversibly recorded in the teeth.

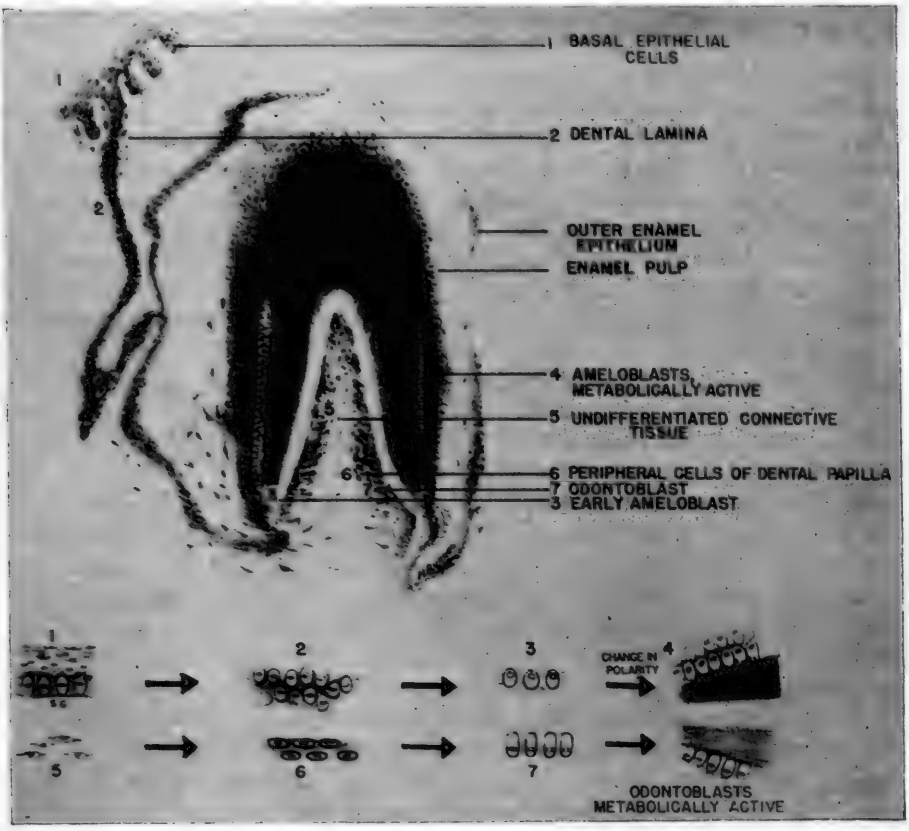

Fig. 1.—Diagrammatic illustration of genesis of ameloblasts and odontoblasts. Tooth bud arises as result of invagination of oral epithelium (*1*), although only enamel portion (*black*) of tooth and its membranous covering are strictly of epithelial origin. These invaginated cells (dental lamina, *2*) proliferate and extend into connective tissue beneath epithelium; they form a somewhat spherical mass at end of lamina; this mass subsequently becomes cup-shaped, exterior of cup forming outer-enamel epithelium. Inner layer of cup (inner-enamel epithelium) differentiates into ameloblast progenitors, the early ameloblasts (*3*) which later change in polarity and become metabolically active (*4*). These cells produce enamel matrix. Connective tissue (*5*), which lies within cup-shaped enamel bud, differentiates peripherally (*6*) into ovoid cells and then into odontoblasts (*7*). Odontoblasts are active in formation of predentin (*white*) which is calcified into mature dentin (*gray*).

The fact that dental development in swine is essentially identical to that of the human being[24] is important, for this permits a more direct evaluation of effects in terms of human tissues.

A survey of radiologic literature reveals that not much attention has been given to dental structures by radiobiologists. One of the earliest to report findings in this restricted field was Leist (1925-1927).[18, 19] He believed that

there might be a direct effect of irradiation on the odontoblasts of teeth with an interruption of dentin formation, that the eruption of teeth was delayed and their roots diminished in growth. Later Herold[17] (1931) repeated Leist's experiment and in general corroborated his findings. Smith[24] (1931) exposed rats' teeth to dosages comparable to those which might be used in x-raying children's teeth and found no ill effects. In the meantime, Blum[12] (1924), Martland, Conlon, and Knef[21] (1925), and Martland[20] (1929) had reported necrosis of the jaws in radium dial painters and related the lesions to destructive action of the ingested radium paint. These studies did not pertain to the teeth themselves, except to the extent which carious teeth might initiate osteoradionecrosis. del Regato (1939)[23] found, from clinical observations of patients who had been therapeutically irradiated, that there are severe carious-like lesions in teeth of patients subjected to large doses of irradiation to the head. He noted the possible causative relationship which an altered saliva may have on these teeth, due perhaps to the simultaneous exposure of the salivary glands with the teeth. Quite recently there have been experiments showing the effects of total body[15] and local[22, 26] x-ray irradiation on rats' teeth, in which enamel hypoplasia, retarded enamel and dentin formation, and slowed eruption of teeth have been reported. Somewhat similar results were found in mice[14] and hamsters.[13] It appears, therefore, that although there have been several valuable contributions in the field of dental radiobiology, there is considerable opportunity to explore basic mechanisms in this field.

MATERIALS AND METHODS

The swine used in this experiment were healthy young animals obtained from the farms of the Bureau of Animal Industry of the United States Department of Agriculture. The animals were raised on diets that were nutritionally adequate in every respect, as follows: (animals weaned at 8 weeks) from 8 weeks to 14 weeks, 70 per cent of the diet was corn, and 30 per cent was a protein-mineral mixture, containing the required proteins, minerals and vitamins for normal growth;* from 14 weeks to 20 weeks, the corn-protein mineral ratio was 75 to 25; after 20 weeks of age, 83 per cent corn was fed and the protein-mineral mixture was 17 per cent. Seven of the swine (5122, 5206, 5215, 5217, 5223, 5224, and 5231) had slight alterations in the source of protein-mineral mixture for 10 weeks after weaning, but not sufficient to affect growth. The weights of the animals at the beginning of the experiment varied between 121 and 154 pounds with a mean of 134.3 pounds. The mean age of

*Protein-mineral mix:

	Per cent
Tankage	10
Fishmeal	10
Soybean meal	35
Linseed meal	20
Alfalfa meal	20
Mineral mix	5

as follows:

	Per cent
Iodized Salt	20.0
Steamed Bonemeal	20.0
Pulverized limestone	57.7
Ferrous sulfate	2.0
Copper sulfate	0.1
Manganese sulfate	0.2

TABLE I

DATA CHART OF FORTY

HOG NUMBER	DOSE (ROENTGENS)	AGE AT EXPOSURE		NUMBER OF DAYS SURVIVAL POST-EXPOSURE	AGE AT DEATH		MANNER OF DEATH	DEGREE OF RADIATION SICKNESS				ORAL LESIONS				DEGREE OF HEMORRHAGE AROUND DEVELOPING TEETH		
		MONTHS	DAYS		MONTHS	DAYS		SEVERE	MODERATE	SLIGHT	NONE	HEMORRHAGE	PETECHIA	ULCERATION	NONE	MASSIVE	PETECHIAL	NONE
232	250-B*	5	20	23	6	13	D		x					x				x
7212	300-B	6		62	8		D		x									x
7092	350-U	5	1	125	9	4	S				x				x			x
6901	350-U	5	7	125	9	10	S				x				x		x	
7312	350-B	5	17	14	6		D	x							x			
6302	400-U	4	15	32	5	16	S			x				x				x
5231	400-B	6	1	13	6	14	D	x										
6612	450-B	4	22	14	5	5	M	x					x				x	
6601	450-U	4	24	12	5	5	M	x					x				x	
6845	450-B	4	10	34	5	14	S				x				x			x
7141	450-B	4	21	11	5	1	D		x						x			
6462	450-B	4	26	12	5	7	M	x					x				x	
7041	450-U	4	29	33	6		S			x					x		x	
41	450-U	4	29	33	6		S			x					x			x
6355	450-U	5	1	34	6	4	S				x				x			x
6231	450-B	5	3	13	5	16	D	x					x				x	
6443	450-B	5	17	11	6		D	x							x			
6043	450-U	6		71	8	10	S				x				x			x
6416	500-B	4	21	11	5	4	D	x					x	x		x		
6383	500-B	4	22	11	5	5	D		x							x		
5826	500-B	4	22	29	5	23	S				x		x		x			

*B = Bilateral.
U = Unilateral.
†S = Sacrificed.
D = Died.
M = Moribund.

Brief record of pertinent facts about animals studied. Equal numbers of bilaterally and unilaterally exposed animals were selected. About 60 per cent of animals were in 450 to 500

the animals at exposure was 5 months, 7 days (157 days), and at death was 6 months, 2 days (182 days). They were bred from Landrace-Duroc-Hampshire-Chester White Stock.

The physical characteristics of the x-ray equipment used in this investigation have been reported in detail.[37] Swine were exposed in groups of 8 at a time to measured amounts of total body x-ray irradiation from a General Electric industrial 2000 KVP, 1.5 Ma. x-ray unit. The calculated half value layer (H.V.L.) for the radial beam which was used was 4.3 mm. Pb. and the roentgens per minute in air administered was 14.7 at a target-skin distance (t.s.d.) of 2 meters. The dosage for the hogs used in this investigation ranged from 250 to 800 r as shown in the chart (Table I). During irradiation the swine were held in cages designed so that excessive movement of animals was prevented, thereby assuring uniform exposure (Fig. 2). Exposures were made

I

ONE IRRADIATED SWINE

HOG NUMBER	DOSE (ROENTGENS)	AGE AT EXPOSURE		NUMBER OF DAYS SURVIVAL POST-EXPOSURE	AGE AT DEATH		MANNER OF DEATH	DEGREE OF RADIATION SICKNESS				ORAL LESIONS				DEGREE OF HEMORRHAGE AROUND DEVELOPING TEETH		
		MONTHS	DAYS		MONTHS	DAYS		SEVERE	MODERATE	SLIGHT	NONE	HEMORRHAGE	PETECHIA	ULCERATION	NONE	MASSIVE	PETECHIAL	NONE
5825	500-U*	4	22	32	5	26	S†				x				x			
6242	500-U	4	24	11	5	7	S	x					x			x		
7311	500-B	5	11	15	5	25	M	x					x			x		
6413	500-B	5	11	19	6	2	M		x						x		x	
5122	500-B	5	26	12	6	10	D		x				x					
6946	500-B	5	27	13	6	9	M		x				x			x		
6751	500-B	6	1	11	6	12	D		x						x	x		
6744	500-U	6	1	19	6	20	D	x					x					
6552	500-U	6	11	15	6	26	M		x				x	x			x	
6281	500-B	6	17	13	7	2	D	x				x	x			x		
6847	550-U	4	18	15	5	2	D	x							x		x	
7223	550-B	5		12	5	12	D	x					x			x		
7231	550-B	5		12	5	12	D	x					x			x		
5743	600-U	4	15	14	4	30	D	x					x					
5746	600-U	4	15	14	4	30	D	x				x	x					
5206	600-U	5	10	14	5	24	D	x				x	x			x		
5223	800-U	5	16	12	5	28	M		x				x			x		
5224	800-U	5	16	12	5	28	D		x						x			
5215	800-U	5	17	13	6		M	x				x	x					x
5217	800-U	5	17	15	6	4	M		x						x	x		

r dose group. Age range at exposure was from 4 months, 10 days, to 6 months, 17 days, making it possible to see progressive developmental effects with increased age in teeth which developed between these limits. Most animals became moribund or died between the eleventh to fifteenth day following exposure. Oral lesions and hemorrhage into dental crypts of developing teeth was a more constant finding above the 400 roentgen dose level. Complete sectioning of all developing teeth undoubtedly would have revealed hemorrhage in crypts of developing teeth in many animals marked "none."

both bilaterally and unilaterally (B and U in Table I) because one phase of the primary experiment was to determine the difference in the $LD_{50/30}$ dose* when irradiation was applied either to one or to both sides of the body. When unilateral exposures were made, the developing teeth were taken from the exposed side of the animal for study.

Animals came to necropsy in 1 of 3 ways: (1) they were found dead at one of the routine inspection periods, (2) they were observed to be in a moribund condition and were, therefore, sacrificed, or (3) they were sacrificed after a period of more than 30 days, as "survivors." As a general rule, animals died of "radiation sickness" between 10 and 16 days after exposure, and animals that lived 4 weeks could be considered beyond danger of succumbing

*Dose lethal to 50 per cent of animals in 30 days.

to irradiation effects. Gross examination was made at necropsy; sections from all lesions were taken for histologic preparation, and routine sections were taken from the tongue and salivary glands; all mandibles were disarticulated and retained for subsequent sectioning of developing teeth. Radiographs were made of each mandible in order to study development and to locate developing teeth. Figs. 3 and 4 are radiographs showing typical development and also the method of marking developing teeth.

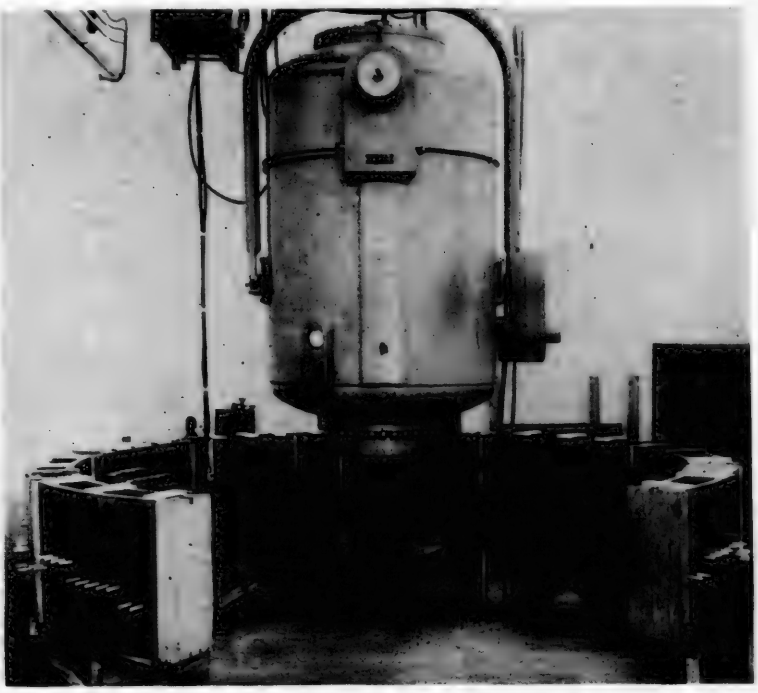

Fig. 2.—Photograph of radiologic setup used in exposing swine at the Naval Ordnance Laboratory, White Oaks, Md. General Electric industrial 2000 KVP, 1.5 Ma. x-ray unit was used to expose swine, at a target-skin distance of 2 meters. The calculated half value layer for radial beam was 4.3 mm. Pb. and 14.7 roentgens per minute in air were administered. During irradiation, swine were held in cages designed so that excessive movement was prevented. Note the adjustable dowl pins projecting through cages, beneath which swine had to remain.

The sectioning of teeth, mandibular bone, and adjacent soft tissues en masse presented some difficulties. In order to retain fully calcified enamel, it was desirable to prepare specimens without decalcifying them, for with decalcification this enamel would be lost. Ground sections of the hard portions of teeth alone, and of bone, have been made by numerous methods,[35, 36, 38, 39, 40] but in order to retain pulps of teeth, bone marrow, and adjacent soft tissue, without preliminary impregnation by hardening substances, a modification of existing methods was devised. Cutting disks, cooled, washed, and lubricated by a water spray, were turned at high speeds (7,500 r.p.m. for 4 inch wheels)

and specimens were moved against the cutting edge. Tissue blocks were retained in a vise, the vise was mounted in the base of a milling machine, and by manipulating several worm gears, desired micromovement of the tissue against the cutting wheels was obtained (Fig. 5). Material could be cut in this fashion immediately following autopsy in sections 0.5 to 1 mm. in thick-

Fig. 3.

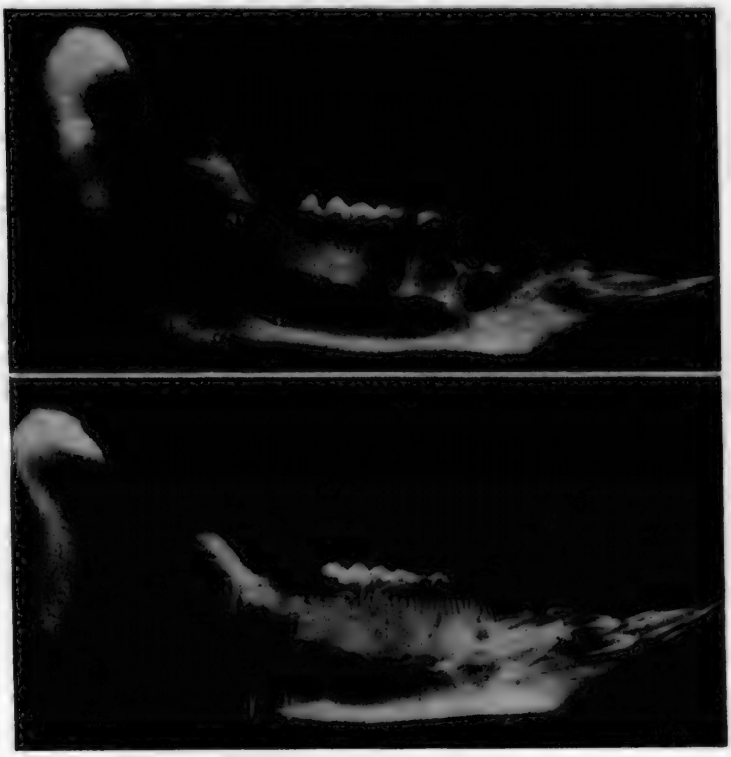

Fig. 4.

Fig. 3.—Radiograph of right half of the mandible of Animal 6612. At age of 5 months, 5 days, this animal showed 1 permanent molar tooth erupted and 1 with only portion of crown developed. Three permanent premolars were developing beneath their deciduous predecessors. Developing permanent canine or tusk tooth and developing incisors could be seen overlapping each other anteriorly. One tooth (first premolar or "wolfzahn") was noted. This tooth characteristically has no deciduous predecessor, but erupts while deciduous teeth are present and remains in dentition.

Fig. 4.—Radiograph of the right half of the mandible of Animal 7311. This animal was 20 days older than that in Fig. 3. It had another molar tooth developing in addition to those described under preceding illustration. Developing teeth were marked by a wire twisted around the mandible. Lingual segment of wire is bent in 2 places to help in radiographic interpretation.

ness. As many as 20 such sections could be made from a hog molar tooth if desired. Sections of this thickness were studied under the research dissecting-type binocular microscope by means of reflected lighting. This permitted a primary evaluation of changes which had occurred, as shown in Figs. 13-15 and 17-22. As sections were cut from the mandibles through the developing

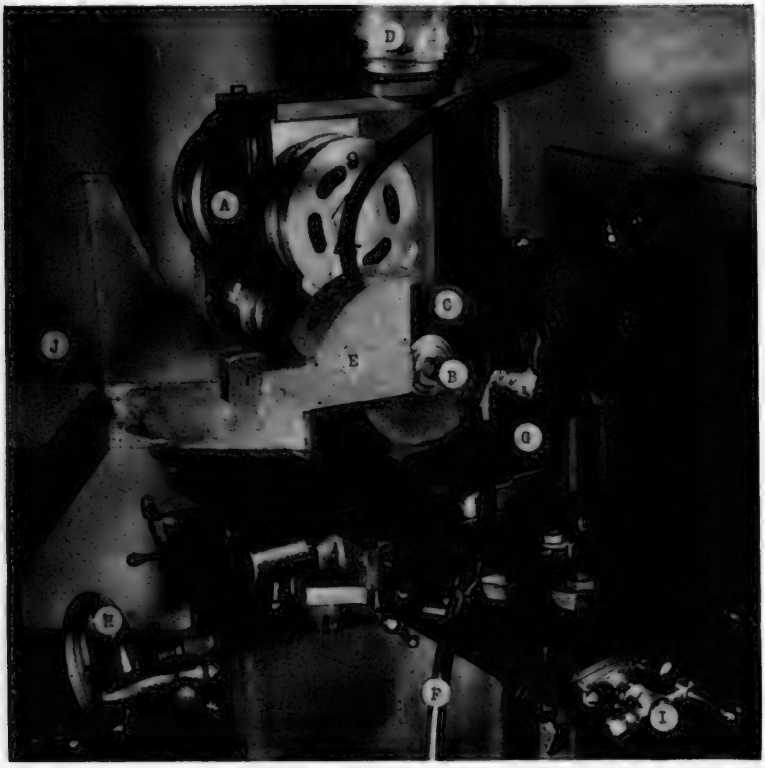

Fig. 5.—High-speed sectioning machine.* This machine was used for making preliminary sections for low-power study and for rapidly surveying large amounts of material. Because of their thinness, the decalcification of sections produced with this machine required only 24 to 48 hours in 10 per cent nitric acid.

A, High-speed motor, 12,000 r.p.m. with full load.

B, Spindle, supporting cutting disk, operated at reduced speed of 7,500 r.p.m. by means or reduction pulley.

C, Cutting disk, hard rubber containing abrasive, diameter 4 inches, 0.0145 inch thick. Sometimes metal circular saw was used, 0.020 inch thick, with 4 inch diameter.

D, Container of water which has rubber feed line to thick felt cleaning and lubricating block housed in guard for cutting wheel. Water lubricates and cools wheels.

E, Guard for revolving cutting wheels, containing felt block. Wheels pass through thin slit cut into felt block, thereby being wiped clean and kept moist.

F, Rubber tube leading from cold water spigot and containing hypodermic needle at its end, through which water is sprayed onto cutting wheels. A second spray is applied to opposite side of wheel.

G, Vise, mounted on table of small milling machine, in which specimen is mounted for cutting.

H, Wheel, controlling cross-drive screw by which specimen is carried slowly against revolving wheel.

I, Crank, operating screw by which mill table can be moved a measured distance, thereby enabling second cut to be made which will produce thin section.

J, Heavy plastic shield behind which operator works.

*This machine is a modification of mechanism developed in the Crystalogrophy Section of the Naval Research Laboratory, Anacostia, D. C., for cutting quartz crystals.

molar teeth it was possible to make preliminary low-power examination of them and determine how profitable it might be to cut additional sections from a given area. At the same time, certain sections could be selected for decalcification, celloidin embedding, and routine procedures for making sections for high-power microscopic study. Since these selected sections were thin, decalcification could be accomplished in 24 to 48 hours.

RESULTS

Oral Mucosa and Tongue

As has been pointed out in the literature,[25] the tissue response within oral mucosa is similar to that of the gastrointestinal tract in general. Petechial

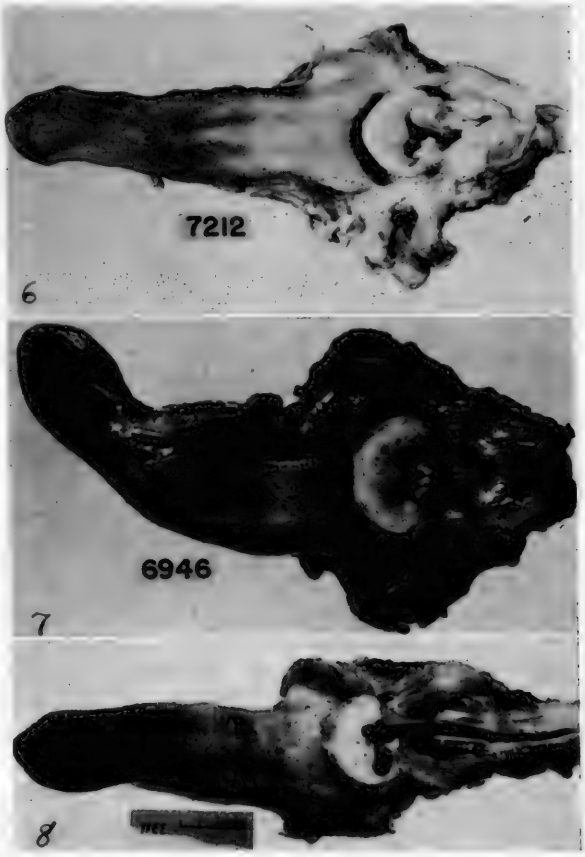

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 6.—Tongue from animal which survived 62 days after receiving a dose of 300 r (bilaterally). Tongue essentially normal in appearance, indicating that there were either no lesions with this dosage, or that repair had occurred. Compare with Figs. 7 and 8.

Fig. 7.—Tongue from animal which succumbed from irradiation sickness in 13 days; dose 500 r (bilaterally). Petechial hemorrhages on all aspects of tongue, on epiglottis, and on tracheo-esophageal trunk. Tonsillar area swollen and discolored by hemorrhage within tissues.

Fig. 8.—Tongue from animal which survived 15 days after receiving 500 r (bilaterally). Hemorrhagic areas on tongue, epiglottis, and tracheo-esophageal trunk. Ulcerated area (4 mm. in diameter and depth) on lateral aspect of the tongue.

and ecchymotic hemorrhages were noted in the oral cavities of 20 of the 41 swine of this investigation. The most common area involved by hemorrhage was in the peritonsillar region. Here there was frequently a marked swelling of the tonsillar lymphoid tissue with a blue-black discoloration of the whole edematous area. Petechiae were also common in the epiglottis and on all surfaces of the tongue (Figs. 7 and 8). The mucosa of the buccal and labial walls of the oral cavity were further sites of petechiae, particularly at the turn of the buccal folds from the gingiva (Fig. 11). Involvement of the gingiva was seen less frequently than any of the above-listed areas, and the hard palate was involved only once in this series—possibly traumatically. Sections from these areas, studied microscopically, showed random hemorrhage, edema, and destruction of the lymphoid elements within the tonsillar mass. In the lymph nodes of the submaxillary region, lymphoid cells were markedly depleted, but reticular cells and erythrocytes showed no damage. Many regions of the tongue contained petechial hemorrhages (Fig. 10) which were probably due to increased capillary permeability.[10] Other routine sections, taken from the tips, mid-portion, and base of the tongue, showed little or no change as far as musculature, nerve endings, or vascular network were concerned. Changes in lingual salivary glands of the mucous variety were in the nature of a decrease in basophilic granules, as described under salivary glands.

Well-demarcated ulcerations were noted in the tongues of 4 of the animals (Fig. 8) while small, shallow, irregular erosions of the mucosal surface were observed in other animals. These lesions may have been due to lessened resistance to damage or to poor recuperative power. In histologic sections (Figs. 9 and 12) these lesions were nonspecific in nature, the only remarkable feature being the markedly diminished leucocytic response. In lesions of 2, 3, or more weeks' duration, hyaline changes in connective tissues, abnormal fibrocytes, and thickening of vessel walls were noted.

Salivary Glands

The study of salivary glands is complicated by the rapid autolysis which occurs if they are not immediately fixed. In nearly all of the swine which were found dead and promptly autopsied, marked necrosis was observed of acini and salivary ducts, and the lumina of ducts were filled with cell fragments and debris. Duct epithelium was frequently partially or entirely missing. In this group of animals there is no way of knowing what changes may have been due to radiation damage.

Changes observed in salivary glands taken from several of the moribund swine and promptly fixed also were suggestive of early autolysis. Some acini were destroyed and others were architecturally disarranged. The damage appeared to be localized in small areas throughout certain lobes, with intervening tissue having a normal appearance. Blood vessels with thickened walls were noted between lobes of salivary glands. The salivary glands of animals which were found in a moribund condition, and in which there was no necrosis of parenchyma or supporting tissue, showed a reduction of basophilic granules within the mucous cells. This was noticed in mucous glands

Fig. 9. Fig. 10.

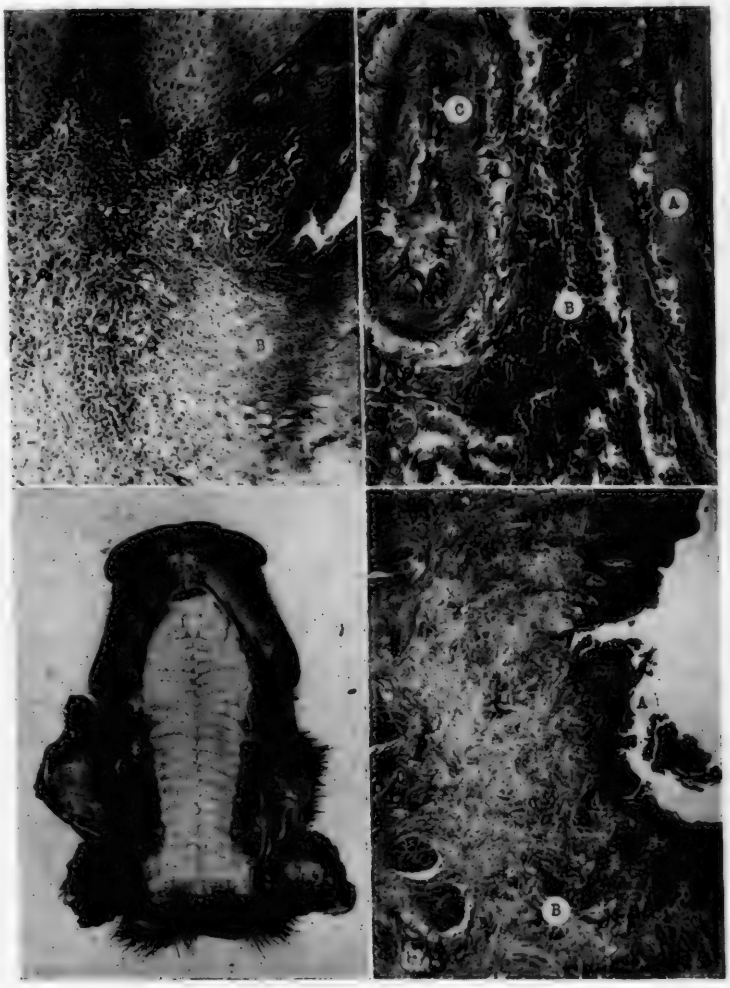

Fig. 11. Fig. 12.

Fig. 9.—Section from ulcer of tongue of Animal 7311 (orig. mag. ×72, 500 r, moribund 15 days). Section from edge ulcer of tongue shown in Fig. 8. *A*, Epithelium which borders ulcerous lesion. *B*, Part of necrotic mass which replaces, epithelium, lamina propria, and part of tongue muscle in ulcerated area. Beneath epithelium and bordering necrotic tissue, infiltration of leucocytes tends to wall off lesion. Leucocytic response is considerably less than usual in lesion of this size in nonirradiated animal.

Fig. 10.—Section from ecchymotic portion of tongue of Animal 7311 (orig. mag. ×240, 500 r, moribund 15 days). Section from tongue, shown in Fig. 8, taken near first muscle fibers (*A*) that occur beneath epithelium. Masses of erythrocytes (*B*) between muscle fibers and arteriole (*C*). Such extravasation of red blood cells is characteristic of tissue damage by irradiation and is a constant histologic finding in many animals in this study.

Fig. 11.—Maxillary portion of hog's head, palatal view, Animal 6552 (500 r, moribund 15 days). Typical petechia and ulcerations which occur in buccal and labial mucosa of hogs which received over 400 r. Such lesions are to some extent spontaneous, but for most part they appear to be extreme reaction to injury. Traumatic injury, which usually would result in no visable damage, results in massive dissemination of red blood cells into tissue spaces in irradiated animals. These are more liable to become infected or undergo degenerative processes, resulting in ulcerations or severe massive inflammatory processes.

Fig. 12.—Section from ulcer of buccal mucosa, Animal 6552 (orig. mag. ×20). (From circled area, Fig. 11.) *A*, Necrosis and exfoliation of portion of the epithelium. *B*, leucocytic response (this is much less cellular reaction than might be expected).

of swine in the 450 to 500 r dose range which were moribund after 11 to 15 days. The effect was to make these cells markedly clear, with less network of precipitated mucigen and cytoplasm, and with an apparent cross sectional enlargement. The nuclei of these cells were smaller and darker staining. Serous glands were not similarly affected.

The salivary glands of swine in the 450 to 500 r group which survived beyond 30 days and were sacrificed showed no necrosis of parenchyma or alteration of basophilic granules within the acini. In some instances there appeared to be an increase of cells between acini, the nuclei of which were similar to the nuclei of mucous glands. Also there were occasional areas of fibrosis, suggestive of scar formation, which contained either no secreting cells or a few shrunken acini. In general, however, there appeared to be recovery from any damage that may have previously existed.

Developing Teeth

The Dental Crypt.—The sections of developing teeth were taken only from the lower molar region of the swine, and usually from just one side of the mandible. Depending on the age of the animals, the second molar teeth* were either unerupted, as shown in Fig. 13, or erupted with still forming root tips as shown in Fig. 14. In the older animals, third molar teeth were also sectioned (Fig. 17). Hemorrhage into the crypts of developing teeth was a common finding, and 15 out of 16 animals that received more than 450 r showed massive hemorrhage in this area (Figs. 17, 19, 20, 21). Frequent hemorrhage into the mandibular canal and marrow spaces was noted (Figs. 17 and 20). If all developing teeth of the maxilla and mandible had been sectioned, undoubtedly more animals would have revealed this response, for, as in the case of petechia of the mucosa, there was no apparent reason why hemorrhages occurred in one area and not another. Although hemorrhage was more common around the developing teeth, there were occasional hemorrhages into the pulp (Fig. 19). Hemorrhages were also found in the pulps of fully developed teeth. There was evidence of residual hemorrhage a considerable time after exposure, as seen at the end of the root in Fig. 14. The celloidin sections (Figs. 23 and 25) verified the extensive bleedings that occurred in these areas. Whether this hemorrhage would have a lasting effect in animals that survived could not be proved by this study, but sections from animals which did live for longer periods (Figs. 14 and 15) suggest that reabsorption would occur without permanent damage.

Changes in the Enamel

The enamel of 4 animals which received less than 400 r (Figs. 13 and 14) was regular in contour, was of a normal thickness occlusally, and gradually became thinner in the cervical area. Architecturally this enamel appeared normal and was similar to that taken from nonirradiated animals. The enamel of the animals in this study which received more than 400 r showed, with few exceptions, areas where there was an alteration of architectural arrangement within the enamel (Figs. 21 and 22), or areas of hypoplasia (Figs. 15, 16, 19, and 23). These lesions occurred in the occlusal third of the teeth. As shown in two

*In this report, the first posterior tooth which erupts without a deciduous predecessor is called the first molar, and the next tooth to develop behind this is called the second molar.[28]

animals which received 800 r (Figs. 21 and 22), the enamel covering the occlusal portion of the teeth was in 2 zones: an inner zone of architecturally regular enamel in normal apposition to the dentin, and an outer layer of rough, irregular enamel. These 2 layers were separated by a sharp line of demarcation which

Fig. 13. Fig. 14.

Fig. 15. Fig. 16.

Fig. 13.—Developing second molar of Animal 232 (orig. mag. ×7). Animal received smallest dose of irradiation (250 r) of any studied. Enamel and dentin were smooth and regular and no lesions were evident in pulp or stellate reticulum. Animal died with moderate signs of irradiation sickness 23 days after exposure at age of 6 months, 13 days.

Fig. 14.—Erupted second molar of Animal 6901 (orig. mag. ×4). Animal received 350 r of x-ray, survived 125 days, and was then sacrificed with no signs of irradiation sickness. Dental hard structures developed normally. Roots not completely developed (age 9 months, 10 days), some engorgement of pulpal blood vessels, and residual signs of hemorrhage around the distal root.

Figs. 15 and 16.—Erupted second molar of Animal 6043 (orig. mag. ×4 and ×7). Two views of same tooth are shown: Fig. 15, a buccal-lingual section through the whole tooth, and Fig. 16, mesial view of crown. Animal sacrificed 71 days after exposure to 500 r of x-ray irradiation. No general systemic lesions noted at necropsy. Hypoplastic lesion involved tip of crown. Yellow-brown discoloration present and thin layer of enamel present over most of hypoplastic area was rough and irregular. Vessels of pulp engorged with blood.

terminated at the amelo-dentinal junction about one-third the distance between the occlusal surface and base of the crown. Below this point the enamel appeared to be regular in contour and normal in thickness and architectural arrangement. In other cases, where there was marked hypoplasia, it was

Fig. 17. Fig. 18.

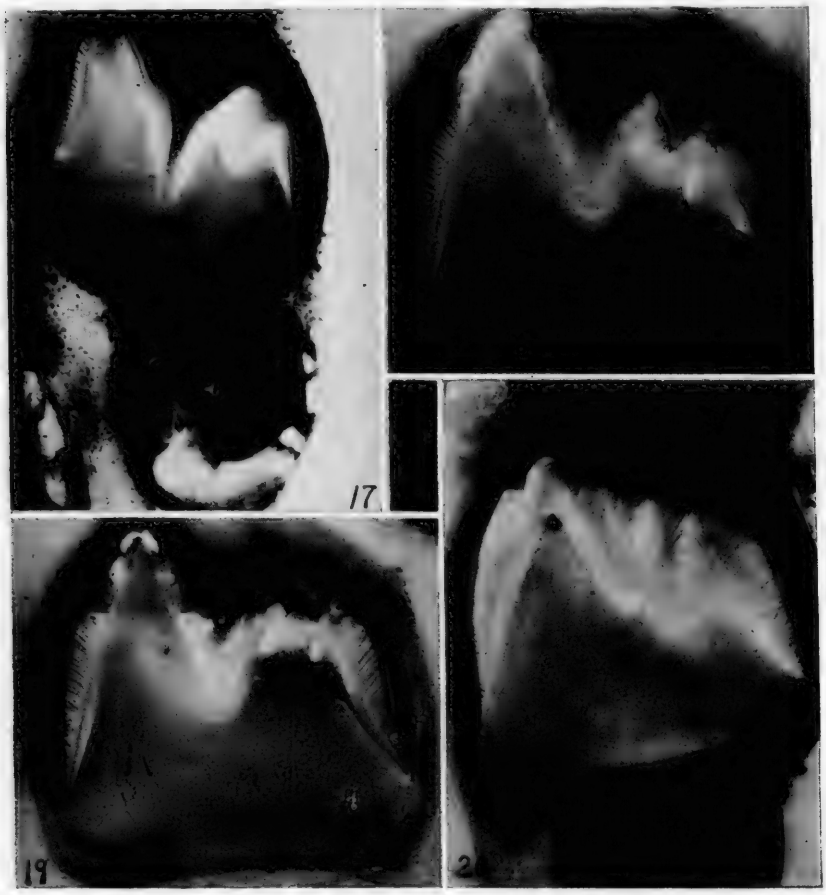

Fig. 19. Fig. 20.

Fig. 17.—Section through third molar and mandibular canal of Animal 7311. Received 500 r (orig. mag. ×7). Pronounced hemorrhage into whole dental crypt and mandibular canal 15 days following exposure to x-ray. No evidence of enamel hypoplasia. Radiograph of this animal (Fig. 4) shows extent of calcification. Radiograph of Animal 6612 (Fig. 3) shows no third molar. Latter animal was 20 days younger than former at death. Probably no enamel had begun to be formed in Animal 7311 at time it was exposed to x-ray (15 days before it succumbed).

Fig. 18.—Developing second molar from Animal 6612. Dose 450 r (orig. mag. ×8). No alteration of pulp or stellate reticulum. Enamel in occlusobuccal area abnormally formed and not as thick as in mid-portion of tooth. Fig. 3 shows stage of development of this tooth, radiographically.

Fig. 19.—Developing second molar from Animal 6847. Dose 550 r (orig. mag. ×7). Hemorrhage into pulpal area, as well as into crypt. Hypoplasia of enamel over tip of the buccal cusp.

Fig. 20.—Developing second molar from Animal 6946. Dose 500 r (orig. mag. ×7). Marked hemorrhage in dental crypt and mandibular canal.

Fig. 21. Fig. 22. Fig. 23.

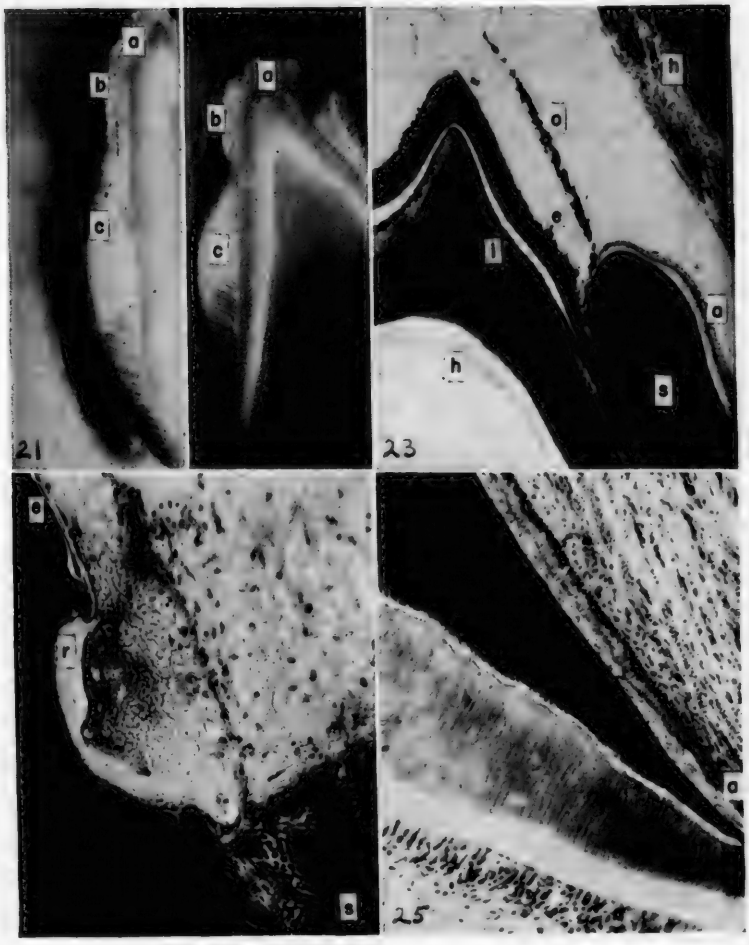

Fig. 24. Fig. 25.

Figs. 21 and 22.—Developing second molar teeth from Animals 5223 and 5224 which received 800 r x-ray irradiation (orig. mag. ×9). Animals same age when exposed (5 months, 16 days) and both lived 12 days after irradiation. Layer of normal-appearing enamel in apposition to dentin over tip of cusp of each tooth (*a*), external to this, there was zone of gnarled enamel (b). Demarcation line between 2 zones formed regular line which would coincide with definite chronologic period in enamel formation. Same ameloblasts produced enamel in zone *a* and zone *b*, but they were functioning differently while producing latter enamel. Enamel which formed inferior to this area of zoning (*c*) was of normal width and the Schreger's lines were regular in arrangement. Hemorrhage into the crypt in Animal 5223.

Fig. 23.—Celloidin section from second molar of Animal 6231 which received 450 r x-ray irradiation (orig. mag. ×35). Thin layer of normal enamel present over occlusal third of tooth. Band of calcified material (*o*) parallel to this primary enamel, but some distance from it in stellate reticulum. No normal-appearing ameloblasts either along this primary band of enamel or along calcified external band. Cells which were found (few on enamel, but predominantly around outer strip) were small, round, or ovoid in shape, and deeply stained. Several isolated round masses of enamel-like material ("enamel pearls") were seen bordering enamel (*e*). Wide band of subsequently formed enamel (*s*) present in mid- and cervical portion of tooth. Normal-appearing ameloblasts border this enamel (*a*). Hemorrhagic effects in crypt and in pulp near odontoblastic layer (*h*). Pronounced imbrication line (*i*) occurred in the dentin at a depth chronologically comparing with cessation of enamel formation in primary strip of enamel.

Fig. 24.—Celloidin section from second molar of Animal 6383 which received 500 r x-ray irradiation (orig. mag. ×250). Resorption of enamel (*r*) with the formation of sulcus containing particulate matter and small round cells. In other places some enameloid material occurred in oval layers (*e*) upon primary deposit of enamel. In random areas, complete second enamel formation, architecturally different from the first layer, found as outer zone (*s*).

Fig. 25.—Celloidin section shown in Fig. 24, cervical area (orig. mag. ×250). Development of enamel and dentin in cervical region of teeth normal, even when marked hypoplasia occurred occlusally. Note erythrocytes in stellate reticulum (hemorrhage).

obvious that there had been cessation of enamel formation over the occlusal tips of the molar teeth. In an animal which survived 71 days following a dose of 450 r (Figs. 15 and 16), there was a thin cap of discolored enamel over the occlusal surface of the tooth, whereas the cervical enamel seemed quite normal. A study of another animal which received 450 r and died in 13 days showed a condition of hypoplasia at an earlier age (Fig. 23). Here, again, there was a thin layer of enamel over the occlusal tip of a molar cusp, as well as a complete lack of normal ameloblasts in this area. Located in the stellate reticulum at some distance from the band of primary enamel and parallel with it, there was a band of osteoid material (Fig. 23, *o*), beginning at the junction of the thin occlusal cap of enamel and a broad band of more cervical enamel. This osteoid strip took a basic stain and was bordered by cells with small, round, dark, basic staining nuclei. The enamel which developed on the cervical portion of the tooth appeared quite normal, and as shown in Fig. 25, *a*, the ameloblasts here were regular in size and distribution.

The cessation of enamel formation, as seen in celloidin sections of some of the teeth, was sometimes irregular, so that mounds of later-formed enamel would occur intermittently along the buccal and lingual surfaces or in the occlusal grooves. Usually the intervening valleys contained only the thin strip of primary enamel. That is to say, certain groups of ameloblasts continued functioning and were normal in appearance, whereas over the void areas only markedly altered ameloblasts, or no ameloblasts, were seen. Occasionally spherical masses of enamel-like material ("enamel pearls") were noted located on the primary strip of enamel (Fig. 23, *e*) or sometimes there were oblong enameloid bodies as shown in Fig. 24, *e*. In some instances there was actually a resorbtion of primary enamel, resulting in cavities containing cells and debris (Fig. 24, *r*).

An inclusion of masses of foreign material was noted in the enamel of several animals. In the enamel of a molar tooth of 1 animal there were 7 such masses, the smallest being about 5 enamel rods in width, and the largest being half a microscopic field at ×440. The material in the inclusion was acid staining and the morphology was suggestive of necrosed cells. The most likely explanation is that certain ameloblasts were rendered functionless and were buried by the matrix produced by uninjured ameloblasts on either side of them.

Dentin

The dentin showed only minimal alteration from the normal. In some celloidin sections there was a pronounced incremental line running over the occlusal tip of the cusps and terminating at the sides of crown in the amelodentinal junction just below the point of termination of the thin occlusal cap of enamel (Fig. 23, *i*). Pulpal to this line there were some irregularities in calcification, indicated by spherical areas of dense acidophylic staining with pale spaces between them.

The Dental Pulp

Most pulpal tissue appeared to be normal; however, in several teeth there was hemorrhage beneath the dentin to the extent that odontoblasts were obscured

in certain areas. No significant morphologic alteration of the odontoblasts was seen.

DISCUSSION

The acute lesions of the oral mucosa, namely, hemorrhage of various degrees and necrosis which often progresses to ulceration, are typical of lesions found in the whole alimentary canal. The mechanisms involved which lead to such responses have been variously explained, some believing that certain direct effects of radiation are important and others attributing many of these changes to indirect processes. The early reduction in the number of white blood cells, first lymphocytes, then granulocytes, is important because of the reduced natural defenses of the organism to bacterial invasion. Soon after irradiation, ectasia of blood and lymph vessels and increased capillary permeability lead to alteration in the blood supply to the various tissues. Hemoconcentration, interstitial edema and hemorrhage, and anoxia may develop. Once the cycle is established it becomes more difficult to restore the normal functions of the involved tissues.[32] Many of the basic responses are unexplained as to pathogeneses, or there are conflicting viewpoints as to the mechanisms involved. There is disagreement, for example, as to whether necrosis of connective tissue and resulting proliferation of connective tissue cells cause the obliteration of blood and lymph vessels, or whether the obliteration of vessels causes the connective tissue changes.[9] Certainly some of the oral lesions are the result of direct damage, for there is considerable evidence that the epithelial lining and secretory elements of the mucosa are among the most sensitive of all tissues to irradiation.[2] The fact that the tissues of the mouth become less sensitive to pressure or pain after irradiation[1] may be a factor in increased traumatic damage in the process of eating, with resulting ulcerations. This thought is substantiated by the predominance of lesions in areas which might have been damaged by the animals' own teeth in chewing. Changes in the salivary glands are comparable to those described in the salivary glands of rats when various "alarm reaction" stimulae were applied.[30] The complexities of the mammalian body are such that it is difficult to determine which responses are due to local effects and which are of a systemic nature[3] when total body irradiation is administered. There is an accumulation of information which permits us to visualize possible mechanisms which may be working together to produce irradiation sickness and its sequellae. It appears possible in the light of recent investigations to differentiate between direct cell destructive action of the rays and those effects brought around indirectly due to circulation of toxic substances released from the cells which disintegrate under the impact of irradiation.[2b] The nonspecificity of reactions, such as hyaline change in connective tissue, thickening of vessel walls, and production of abnormal fibrocytes, makes it impossible to attribute them individually to irradiation damage; however, they are usually associated with chronic or delayed radiation reactions.[7]

Several interpretations as to the cause of the enamel hypoplasia in these swine may be made. Perhaps the first question is whether such lesions need necessarily be due to irradiation. A study of teeth from nonirradiated swine

raised on balanced, nontoxic diets revealed no lesions similar to those described. Swine raised on deficient diets[31] or on diets containing toxic substances[29] may develop hypoplastic deficiencies. Just as in the human being, it is logical to assume that hypoplasia of the enamel in the teeth of swine may occur as a result of various stimuli, and there are no clear-cut differential characteristics between the lesions arising from different causes. Heilbrunn and Mazia[4] have observed that the nature of protoplasm is such that it is sensitive to various types of external agents, and the response may be much the same regardless of the diversity of treatments. Many physiologic responses to irradiation are practically identical to the effects produced by other injurious agents whose mode of action is wholly unlike that of radiation.[5] The final morphologic and architectural alterations may give little clue as to the preceding mechanisms which caused them.

In radiation hypoplasia, morphologic alteration of ameloblasts (or their actual disappearance) is observed in many developing teeth. There are several reasons or parallel situations explaining why certain ameloblasts might be altered by ionizing irradiation. In the first place, ameloblasts are epithelial in origin, and as such they come high in the list of radiosensitive cells. Second, it seems that those ameloblasts which are metabolically active are most affected, whereas ameloblasts which differentiate later on in the cervical portion of the teeth look normal and form normal enamel. This too follows the pattern of cellular reaction elsewhere, for cells in close relationship are found to show marked variation in radiosensitiveness,[11] and the same cell may be more or less resistant depending upon its state of development and metabolic activity.[6] As was pointed out in the introduction and in Fig. 1, a change in polarity normally occurs in ameloblasts just prior to formation of enamel matrix. This may be a significant change as far as radiosensitivity is concerned, just as it is functionally, for those ameloblasts which have not yet changed in polarity appear to be unaffected by radiation. Irradiation may disrupt the process of polarity change in time sequence or numbers sequence in somewhat the same fashion as loss of polarity occurs in chondroblasts after irradiation.[8] It seems likely that the ionizing effect of irradiation on the ameloblast causes functional changes or destroys cellular enzymes with the result that normal metabolic processes are deranged.[5] This results in failure of enamel matrix formation. If the cells are affected minimally, the ameloblasts may function in an abnormal fashion and form altered enamel as seen in Figs. 21 and 22. If affected greatly, necrosis and disappearance of vulnerable ameloblasts may occur, with no enamel matrix formation (Figs. 23 and 24).

SUMMARY

Oral tissues, salivary glands, and developing teeth of 41 young swine were studied grossly and histologically to observe pathologic changes resulting from x-ray irradiation. These swine were selected from 120 animals which were exposed to 2000 K.V. total body x-ray irradiation in doses of about 250 to 800 roentgens in air. A hard-tissue sectioning machine was developed for making

sections through undecalcified teeth and jaws. Gross changes in the oral mucosa consisted of petechial and ecchymotic hemorrhages in 49 per cent of the animals and marked ulceration in 9.8 per cent. Histologic study revealed irregular erosions of the mucosa in about 30 per cent of the animals and either petechial or massive hemorrhage around developing teeth of 70 per cent of the swine. Regional lymph nodes showed a high degree of sensitivity to x-ray irradiation by exhibiting hemorrhage and depletion of lymphoid cells. Salivary glands were among the less sensitive tissues, with regard to morphologic change; however, a definite decrease of basic staining granules was observed in acute stages of radiation sickness, with complete recovery in 30-day survivors. A characteristic kind of hypoplasia of the enamel of developing molar teeth was observed among swine which received more than 400 r. Ameloblasts actively producing enamel matrix appeared to be sensitive to irradiation. Other portions of the teeth showed minimal effects.

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SOME OBSERVATIONS ON DENTAL CARIES IN CENTRAL CHINA

D. AFONSKY, D.D.S.*

Changsha, Hunan, China

IT HAS been stated[1, 2] that the Oriental diet, rich in carbohydrates, is inducive to dental caries. The advocates of the nutritional theory of caries claim that adequate diet is a prerequisite for immunity to caries. Since malnourishment is common in China and the diet used is rich in carbohydrates, the caries rate in this country should be very high. However, clinical evidence is at variance with both above statements.

MATERIAL AND FINDINGS

The present survey was undertaken in Changsha, the capital of Hunan province (Central China). It includes the students of 8 schools, totaling 3,349 persons. The majority (2,911) were natives of Hunan, the rest being representatives of 20 provinces, and 2 of overseas origin (Java and Trinidad). They lived in Hunan from 2 to over 5 years. The age varied between 12 and 24 years in the 1,650 girls and between 12 and 27 years in the 1,699 boys.

Of the 95,830 permanent teeth examined, 2.46 per cent were found to be affected by caries, or an average of 0.7 tooth per person. Caries was present in 28.4 per cent of the students, 71.6 per cent having no active caries, missing teeth as the result of caries, or restored teeth (D.M.F. negative persons). Among those with caries, 39.2 per cent had only 1 D.M.F. tooth per person, 30.0 per cent had 2 D.M.F. teeth per person, and 13.0 per cent had 3 D.M.F. teeth per person. Only 2.3 per cent of those examined had over 6 D.M.F. teeth. Although the per cent of individuals having 1 or more carious teeth is influenced by age, the significance of this observation becomes evident on comparison with conditions in other countries (Table I).

TABLE I

PERCENTAGE OF SOUND TEETH AND PERCENTAGE OF PERSONS HAVING ONE AND MORE D.M.F. TEETH AMONG CHINESE AND ENGLISH STUDENTS

COUNTRY	AGE	SOUND TEETH (%)	PERCENTAGE OF PERSONS WITH: 1 TO 4 CARIOUS TEETH	5 TO 8 CARIOUS TEETH	OVER 8 CARIOUS TEETH
China	13-27	97.54	89.2	10.7	0.1
England*	16-18	6.4	37.3	32.6	23.6

*British Dental Association as quoted by Kurt H. Thoma.[16]

It was discovered that caries did not necessarily develop at an early age, but might start at any age under observation. Many of the students examined had their first caries experience at 19 years of age or even later. The caries rate often did not increase with the age of the person. Some students developed caries in only 1 tooth, predominantly in one of the lower first molars, at the

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*Present address: University of Rochester, Rochester, N. Y.

age of 12 or 13 years without any increment in later life. The percentage of persons suffering from caries and percentage of D.M.F. teeth in different age groups are presented in Table II.

TABLE II

PERCENTAGE OF PERSONS WITH CARIES AND PERCENTAGE OF D.M.F. TEETH ACCORDING TO AGE

AGE	NUMBER OF PERSONS	NUMBER OF TEETH	PERCENTAGE OF PERSONS WITH CARIES	PERCENTAGE OF TEETH D.M.F.
13	283	7,689	21.5	1.8
14	406	11,254	20.7	1.8
15	409	11,484	24.9	2.1
16	567	15,833	28.2	2.3
17	622	18,019	29.3	2.5
18	445	12,763	28.5	2.1
19	292	9,201	31.5	2.3
20	118	3,482	33.0	2.8

Of all the carious teeth 83.4 per cent had only 1 surface involved. The first and second molars accounted for 93.7 per cent of the D.M.F. teeth (the lower first molars alone accounting for 45.7 per cent),* premolars 3.4 per cent, third molars 1.4 per cent, upper incisors 1.3 per cent, and lower incisors 0.2 per cent. In none of the cases examined was caries of the canines seen. No mottling of the enamel was observed.

It has been shown[3, 4, 5] that the caries attack rate in China has marked geographic variations, being lowest in Mongolia and highest in Southern coastal regions (Kwangtung). Our findings are in agreement with these observations (Table III).

TABLE III

COMPARISON OF CARIES ACTIVITY IN HUNAN AND KWANGTUNG

PROVINCE	PERSONS EXAMINED (NO.)	PERSONS WITH CARIES (%)	MAXIMUM NUMBER OF D.M.F. TEETH PER PERSON	AVERAGE NUMBER OF D.M.F. TEETH PER PERSON	D.	M.	F.
Hunan	2911	26.0	6	0.6	0.53	0.03	0.04
Kwangtung*	40	75.0	11	4.3	1.27	1.15	1.88

*Kwangtung has the highest caries rate in this survey.

Since the majority of the students were natives of Hunan the following discussion deals mainly with conditions prevailing in this province. Of 2,911 Hunanese examined, 26.0 per cent had caries experience. Only 2.1 per cent of all the teeth were found to be affected, or an average of 0.6 of a tooth per person. The female students showed a slightly higher incidence of caries than the boys. There was also slight variation between the students natives of Changsha and other districts of Hunan (Table IV). Although statistical data

*Lau[3] stated that among the natives of Kwangtung 90.54 per cent of all carious teeth were molars.

for the rural population are not available, there is strong evidence supported by many years of experience in this part of China that the caries attack rate among the farmers is still lower than the 26.0 per cent determined for students.

No cases of acute or rampant caries were observed. This type of caries is unknown in permanent teeth in this part of China, though it does affect the deciduous teeth, which cases give a history of constant use of candy "pacifiers." Gingival caries is also extremely rare in young persons. This type of caries, and also proximal surface caries, is more common in individuals past middle age. When present, it is usually seen in association with evident food impaction due to recession of gingiva or malposition of teeth. In young individuals caries starts, as a rule, in pits and fissures. In this survey, 83.1 per cent of the cavities in the lower first, and 93.3 per cent of the cavities in the lower second molars occurred in the pits and fissures of occlusal surfaces.* The bitewing radiographs for detection of proximal caries of permanent teeth in young Chinese are not nearly as important as they are in young people in the Western countries. The buccal pits of the lower molars and the lingual pits of the upper molars are also places of predilection. No caries of the incisors and canines were observed among the Hunanese.

TABLE IV

INCIDENCE OF CARIES AMONG BOYS AND GIRLS, NATIVES OF CHANGSHA AND OTHER DISTRICTS OF HUNAN PROVINCE

PLACE OF BIRTH	NUMBER OF CASES		NUMBER OF TEETH		CASES WITH CARIES (%)		D.M.F. TEETH (%)	
	BOYS	GIRLS	BOYS	GIRLS	BOYS	GIRLS	BOYS	GIRLS
Changsha	530	692	15,100	19,796	24.2	30.1	1.93	2.4
Other districts	942	746	26,365	21,823	23.1	26.6	1.77	2.38

The author followed cases of pigmented pits and fissures for periods of from 2 to 6 years without observing any clinical evidence of progression of the process. In such cases, on grinding, the involved dentin is usually found to be of the hardness of normal dentin, but pigmented a deep brown with a light brown or yellow zone at the periphery. The pigmented part is conical in shape and may reach from 2 to 3 mm. in depth. As a rule, in Hunanese, caries is very slow in its progress, and big accumulations of soft and leathery dentin are conspicuously absent in occlusal cavities.

DIETARY SURVEY

The fact that all 3,349 students attended boarding schools simplified considerably the procedure of collecting the dietary history. They received their food from their respective kitchens 3 times daily. The individual diet only varied in the amount consumed and supplements. Three schools were selected as representing the poorest, medium, and highest regular diets received, and the average consumption per student was calculated (Table V). Additional

*Lau[3] cited 86.87 per cent for the similar type of cavities in all molars among the natives of Kwangtung.

forms were distributed among the students for supplemental diets in which all the foodstuffs consumed besides the regular diet were entered. The supplemental diets were further subdivided into 3 groups and the percentage of students consuming different types of diets was calculated (Table VI). From the data of the regular and supplemental diets the maximum diet in the 3 schools, respectively, was obtained (Table VII).

TABLE V

REGULAR DIET IN THREE SCHOOLS EXPRESSED IN GRAMS PER CAPITA PER DAY

SCHOOL	RICE	CHINESE CABBAGE	GREEN VEGETABLES	SOY BEANS	FAT AND VEGETABLE OIL	FISH	EGGS	MEAT	BEAN CURD
I	450.0	151.0	68.0	8.0	18.0	67.0	15.5	23.0	33.0
II	450.0	144.0	70.0	-	11.5	14.0	-	16.0	54.0
III	450.0	308.0	209.0	-	19.0	-	-	*	45.0

*Meat and fish were served in amounts of 1 ounce twice a month.

Subdivision of supplemental diet:

Type I.—Meager: an occasional ounce of peanuts, an orange, and a few candies.

Type II.—Average: a cup of milk, peanuts, an egg, and a piece of bread once in a while.

Type III.—Highest: eggs, milk, butter, bread, and peanuts more or less regularly.

TABLE VI

PERCENTAGE OF STUDENTS CONSUMING DIFFERENT TYPES OF SUPPLEMENTAL DIETS

SCHOOL	NO SUPPLEMENTAL DIET (%)	MEAGER SUPPLEMENTAL DIET (%)	AVERAGE SUPPLEMENTAL DIET (%)	HIGHEST SUPPLEMENTAL DIET (%)
I	18.3	17.5	35.9	28.3
II	24.1	25.9	43.7	6.3
III	18.2	23.3	50.1	8.4

The staple diet consisted primarily of carbohydrates derived from crude polished rice and vegetables (Table VIII). Even the maximum diet consumed by a small percentage of students hardly reached the normal daily requirements. Minerals were low in the majority of cases. The "tooth-building" and vitamin D-rich foodstuffs were practically absent from the common diet. Milk and milk products are not used by farmers and vast majority of city dwellers. The students drinking milk (in small amounts) were found to have more carious teeth than individuals who never use it at all. The natives of Kwangtung, having plenty of sunshine nearly all the year round, have a much higher incidence of caries (90.8 per cent) than people of Central China (26.0 per cent) who hardly receive any sunshine during the long winter months. They also consume more sea foods and fish containing vitamin A and D.

No sugar in any form or sweets were provided in the regular diet. Nearly one-half of the students (47.3 per cent) had practically no sugar in their supple-

TABLE VII

COMPARATIVE VALUES OF REGULAR AND MAXIMUM DIETS IN THE THREE SCHOOLS*

SCHOOL	TYPE OF DIET	CALORIES	PROTEINS (GM.)	CALCIUM (GM.)	PHOSPHORUS (GM.)	IRON (MG.)	VITAMIN A I. U.	THIAMIN (MG.)	RIBOFLAVIN (MG.)	NIACIN (MG.)	ASCORBIC ACID (MG.)
I	Reg.	1583.5	84.0	0.643	1.35	14.86	3170	2.06	1.130	6.038	109.0
	Max.	2363.0	114.28	0.796	1.836	16.95	3869	2.766	1.519	11.308	109.6
II	Reg.	1547.5	78.0	0.599	1.13	10.97	2905	1.24	1.0	4.170	88.0
	Max.	1954.0	97.30	1.327	1.99	11.94	3053	1.435	1.217	7.853	88.6
III	Reg.	1499.0	59.8	0.770	1.08	11.20	5540	1.29	0.96	4.970	199.0
	Max.	2142.0	107.96	0.846	1.662	14.12	6359	2.366	1.277	12.361	199.0
Normal requirements†		3800.0	100.0	1.4	‡	15.0	6000	1.7	2.5	17.0	100.0

*The diet is assessed according to dry weight of foodstuffs. Cooking losses are not ascertained.
†Recommended daily allowance for boys of 16 to 20 years of age, Food and Nutrition Board, National Research Council. Revised, 1948.
‡Phosphorus allowance should be approximately 1.5 times that for calcium.

TABLE VIII

PERCENTAGE OF CALORIES DERIVED FROM CARBOHYDRATES, PROTEINS, AND FATS IN REGULAR DIET

SCHOOL	CARBOHYDRATES	FATS	PROTEINS	% OF PROTEINS OF ANIMAL ORIGIN*
I	71.0	15.9	13.1	49.0
II	82.0	8.0	10.0	25.0
Standard requirement	50-60	30-40	10-15	

*In some of the schools meat and fish were served in amounts of 1 ounce per person twice a month.

mental diets; 24.5 per cent averaged a daily intake of 10 to 15 Gm., preferably as candy; 23.4 per cent used about 50 Gm. a day, and the rest 4.8 per cent over 50 Gm. a day, the highest consumption being 120 Gm. in 2 cases. There was also absence of easily fermentable carbohydrates, juices, beverages, and sticky foodstuffs capable of adhering to the tooth surface.

Refined foodstuffs, such as sugar, candy, and flour, are almost entirely absent in common diet in this part of China, the foods consumed being "natural." The only preserves used are homemade dried and pickled vegetables (low ascorbic acid content). Sugar cane, chewed raw, is a luxury indulged in by young and old alike.

The Chinese have customarily 3 meals a day, or even 2 (especially during the last war) and no food is usually eaten between meals. Tooth brushing is exercised by many, though students brushing their teeth 2 or 3 times daily often develop more cavities than those who may never use a toothbrush and still remain free from caries. Rinsing of the mouth with water after each meal is a common practice. It seems that the effect of tooth brushing may be expected to be more beneficial in cases of diets rich in easily fermentable carbohydrates and sticky foodstuffs.

DISCUSSION

The low incidence of caries among the Chinese of Central China, involvement of single teeth in otherwise caries-free persons, striking prevalence of persons with only 1 tooth affected, freedom from caries of the anterior teeth, slowness of the process associated with small amounts of soft and leathery dentin, absence of acute or rampant caries, and predominance of involvement of teeth with morphologic defects (pits and fissures) indicate considerable immunity to caries of the natives of this part of China.

It has been repeatedly stated that an adequate diet comprising all the essential nutrients is an all-important factor in the immunity to caries. However, there is no conclusive evidence that an adequate, optimal, or balanced diet would influence the caries attack rate.[6] From several observations,[7-12] it appears that caries is more prevalent in well-nourished than in malnourished individuals. Although, as judged by the standards of the Food and Nutrition Board, Na-

tional Research Council, the overwhelming majority of the students examined were malnourished, the caries attack rate was found to be significantly lower than in well-nourished people of other countries.

Carbohydrates have been blamed most often for the initiation of the carious process as substances providing an easily fermentable substrate which leads, through bacterial activity, to formation of acids on the surface of the teeth with subsequent decalcification of the enamel. Diets high in carbohydrates have been considered predisposing to caries. If this be true, the Oriental diet, supplying as much as 82 per cent of the calories from carbohydrates, predominantly rice which gives an acid residue,[1, 2, 15] should predispose to rampant caries. In fact, it has been stated[1] that Polynesians eating taro (alkaline residue) have only 4.59 per cent of persons affected by caries while Polynesians eating rice have an incidence of caries 98.5 per cent, the inference being that the Oriental diet, rich in carbohydrates (rice), was responsible for the high caries rate. Our observations carried out among the Chinese consuming this type of diet disprove the validity of this statement. On the contrary, it seems beneficial in the production of caries immunity. In so far as it has been demonstrated[6] that the restriction of sugar either refined or natural is effective in the control of dental caries, we are prone to agree with the observers who assert that it is not the amount of carbohydrates consumed, but their type (mono- or disaccharides) and the degree of their refinement that play the important role (Jay,[20] Fosdick,[19] Collins, Jensen, and Becks[18] and Stephan[21]). Our observations on children support this assumption.

Chinese immune to caries do not develop caries during their sojourn in Western countries subsisting on Western, native, or mixed diets from 2 to 8 years. None of over 50 persons examined between 20 and 45 years of age developed caries while abroad. Disinclination of many of them to certain foods supposedly conducive to caries, such as sweets, cakes, beverages, etc., should be borne in mind.

It has been stated that a diet rich in animal proteins and low in carbohydrates is inducive to dental health (Eskimos). That the reverse is also true is shown by this survey.

Low intake of calcium does not seem to predispose to caries. In our survey, the low consumption of minerals was associated with low caries rate. The same phenomenon is observed in cases of repeated pregnancies followed by prolonged periods of lactation (exceeding well over a year) in women subsisting on low mineral diets in this part of China. While caries is uncommon in such instances, periodontal disease is rampant. There is no evidence that children born of such mothers are predisposed to caries.

It is of interest to note, that in diabetics examined (18 persons), the incidence of caries was very negligible (1 person), while diffuse periodontal disease was present in every case.

It has been mentioned above that Chinese from southern coastal provinces are more susceptible to caries than natives of Central China. Most of those examined lived in Hunan from 2 to 5 years. How many cavities they might

have developed had they lived in their respective provinces can only be conjectured. Lau[3] stated that the incidence of caries among the natives of Kwangtung is 90.8 per cent against 75.0 per cent among those residing in Hunan as determined in this survey. It appears that the local diet, although low in essential nutrients, does not predispose to caries. It is pertinent to note that the southern provinces, coincidentally, have had the strongest influence of Western civilization over a long period of time. The reverse is true of Mongolia with extremely low caries incidence. High consumption of fat by Mongols has been held responsible for their immunity.[4]

Lack of detailed dietary surveys precludes evaluation of dietary factors in other parts of China. Differences in dietary habits, mode of preparation of food, consumption of refined carbohydrates, and variations in fluorine content of water and foodstuffs in different provinces may play an important role. Vitamin B complex intake may also be of significance.[13, 14]

CONCLUSIONS

The Oriental diet rich in carbohydrates (rice) does not predispose to caries. On the contrary, it has been found that such diet is associated with considerable immunity.

Adequate diet is not essential for immunity to caries. Caries is less common in malnourished than in well-nourished individuals.

The local effect of foodstuffs and dietary habits appear to be of greater importance in susceptibility to caries than the nutritional status of the person. Lack of easily fermentable carbohydrates and sticky foods, frequency of meals, and absence of eating between meals may be considered as major factors in immunity.

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BILATERAL SYMMETRY OF DENTAL CARIES

M. G. WHEATCROFT, L. J. GERENDE, C. A. SCHLACK, B. L. TAYLOR, V. J. BERZINSKAS, AND C. E. MULLINS

Naval Medical Research Institute, Bethesda, Md.

INTRODUCTION*

INVESTIGATORS in the field of dental research have assumed as a basis for their experimental work that the caries incidence in the right and left quadrants of the mouth is exactly the same.[1, 2, 3] A review of the pertinent literature reveals a number of observations regarding the occurrence of bilateral symmetry of dental caries incidence.

Day and Sedwick[4] noted symmetry of bilaterally paired surfaces and further observed the anatomic structure and position of tooth surface were important factors in caries incidence. Hyatt[5] showed symmetry by comparing percentages of involved paired surfaces in the right and left quadrants. Knutson and Klein[6] showed bilaterality by summing the missing teeth for each quadrant. Cheyne and Drain[7] compared totals of missing first molars of each quadrant and reported symmetry. Montelius[8] compared totals of paired missing teeth, paired carious teeth, and teeth indicated for extraction and stated "the difference between the right and left side is small, probably no significance." Strusser and Dwyer[9] and Leigh[10] also have reported caries occurring with bilaterality. Betram and Brown[11] studied the bilaterality of pairs of teeth regarding caries incidence and reported that the occurrence of caries in pairs more than tripled the requirements for statistical significance. They also presented a method of statistical analysis which is easily understood.

Cohen[12] found a difference between sides of the mouth in caries incidence, basing his findings on the total teeth affected. Brekhus,[13] Scott,[14] and Driak[15] reported caries incidence to be slightly higher on the left side of the dental arch. Scott[14] studied 2,400 bilaterally situated posterior teeth by means of bite-wing radiographs and observed 73.1 per cent were involved bilaterally and that 26.9 per cent were affected unilaterally. Brucker[16] made comparisons between right- and left-side incidence by comparing totals of the two sides. He stated, "Bilateral caries which is supposed to be rather constant was only partly demonstrated in the white children."

Losee,[17] studying only the mesial, occlusal, and distal surfaces, reported that in 11,513 bilateral pairs of these surfaces, 3,974 pairs (34.5 per cent) showed caries in both of the corresponding surfaces, and that 2,437 (21.2 per cent) involved only one of the paired surfaces. If we consider only those pairs of surfaces which showed incidence of caries in either one or both sur-

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faces, we have a total of 6,411 pairs of such surfaces and the rates or pairs showing bilateral symmetry to this total is approximately 62 per cent. If we consider the ratio of the total number of pairs showing bilateral symmetry of caries or noncaries to the total number of pairs of surfaces, 78.8 per cent showed symmetry. Thus, in Losee's data, whatever the manner in which we approach the question of symmetry, we find an appreciable degree of symmetry and also a significant degree of asymmetry.

In a recent study of more than 20,000 proximal tooth surfaces Barr[18] found bilateral symmetry to exist as a characteristic of the aggregate, but found that it was seldom encountered in the individual.

Some previous work dealing directly or indirectly with the problem of bilateral symmetry was based on the summation of caries incidence separately for each quadrant. These sums were then compared to determine if they differed materially. The relation of anatomic structure of the tooth and tooth position was not considered, and their role, if any, in caries incidence was more or less ignored.

It seemed to the writers that, in measuring bilateral symmetry, the probability of the occurrence of caries might be affected by structure and position of the teeth, and hence that from tooth to tooth, or from surface to surface, or even from groove to groove, the rate of caries incidence might vary considerably. Furthermore, the rates of incidence might differ in bilateral pairs to such an extent as to account for the discrepancies introduced by an assumption of perfect symmetry. And if this were true, then the method of measuring bilateral symmetry by quadrant summation and comparison of total caries incidence might introduce discrepancies if one or more teeth on either or both sides were missing. For, if the frequency of caries varies in the manner supposed, then missing teeth not bilaterally paired might make for an appreciable difference in the frequency of caries in the quadrants.

These considerations led us to gather experimental data regarding bilateral symmetry. The use of data on human beings was desirable but not feasible. However, since Cox, Dodds, Dixon, and Mataschak[19] had noted, in passing, the occurrence of a degree of bilateral symmetry in white rats, it was decided to use our caries-susceptible strain of these animals. The fact that highly accurate determination of the presence of caries is possible in the white rat gave further reason for the use of these animals.

We posed the following questions:

A. Is there a bilateral symmetry of caries incidence between pairs of grooves in the molar teeth of a caries-susceptible group of white rats?

B. If there is such bilateral symmetry, how can it be determined?

PROCEDURE

The data of this study were derived from two groups, all the members of which were drawn from the same controlled caries-susceptible colony of white rats.

The first group of experimental animals comprised 255 weanling white rats, 114 males and 141 females. This group was maintained for 100 days

on the cariogenic diet of Hoppert, Webber, and Canniff[20] (altered by the substitution of coarse yellow hominy grits* for ground whole corn), and containing varying amounts of estrogen[21] in some cases, methyl testosterone[21] in some others, and varying amounts of oxalates[22] in water in the remainder. The second group of weanling white rats (50 males and 50 females) was drawn from the same controlled colony, and was fed the altered dietary of Hoppert, Webber, and Canniff, referred to previously, without the addition of test substances. Conditions were constant for all the animals with the exception that the two groups were not all on experiment at the same time.

All the animals were sacrificed after 100 days on the various dietary regimen. The jaws were prepared for study, and the teeth in each jaw ground in occlusocervical planes until the pulps were exposed. All examinations were made under a stereoscopic microscope at a magnification of ×10.5 and ×40.[23] All the grinding of teeth and recording of findings was done by one of us (B. L. T.), keeping the interpretation variable at a minimum.

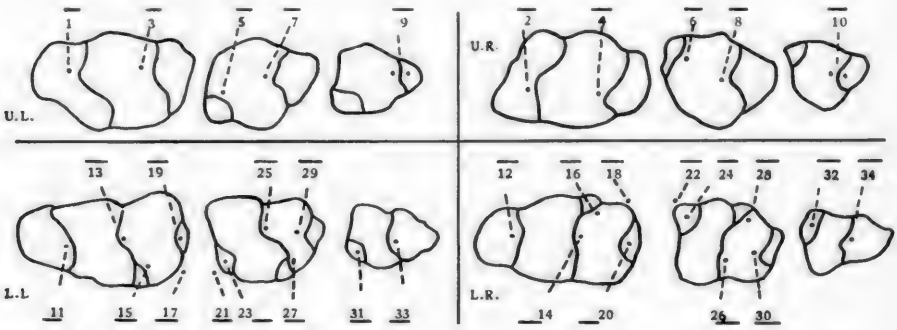

Fig. 1.—Groove positions in the molar teeth of white rats. Numbers 17, 18, 21, and 22 designate smooth surface areas which have been called grooves for convenience.

Each group was examined separately, comparisons being made directly between bilateral paired grooves within bilaterally paired molars (Fig. 1).

The object of dealing with the groups separately was to determine first if the variation in diet affected symmetry. If no material differences between the groups were exhibited, then it was proposed to lump the two groups together so as to determine comparatively stable rates in regard to the question of symmetry and asymmetry.

RESULTS AND DISCUSSION

It is obvious that the use of animals in a study of bilateral symmetry has the advantage of making possible the control of some variables which may be associated with caries incidence. We refer to such variables as: (1) tooth brushing habits; (2) hereditary differences; (3) diagnosis of caries; (4) cariogenic time factors; (5) dietary variation, and (6) environmental (temperature, humidity, housing, isolation, and vermin).

We suppose, then, that in a controlled study some of the factors which may be associated with caries incidence are eliminated. It seems reasonable

*Manufactured by Quaker Oats Company, Chicago, Ill.

further to suppose that if bilateral symmetry of caries and of noncaries occurs in the white rat, then a controlled study would result in demonstrating at least as high an association between pairs of molar grooves, if not a higher degree, as in uncontrolled animals (human beings). With these suppositions in mind, we may now proceed to examine the data obtained from our study.

TABLE I

INCIDENCE OF CARIES BY GROOVES AND INCIDENCE OF SYMMETRY OF CARIES AND NONCARIES BY PAIRED GROOVES

Uniform Dietary Group (50 Males and 50 Females)

GROOVES	CARIES INCIDENCE			INCIDENCE OF SYMMETRY OF CARIES AND NONCARIES		
	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL
1	17	23	40	29	34	63
2	22	25	47			
3	32	30	62	29	34	63
4	29	24	53			
5	8	13	21	41	39	80
6	9	6	15			
7	29	26	55	30	40	70
8	21	26	47			
9	9	8	17	40	45	85
10	3	5	8			
11	12	13	25	35	40	75
12	17	15	32			
13	45	48	93	45	48	93
14	48	50	98			
15	35	40	75	38	41	79
16	33	39	72			
17	18	21	39	37	36	73
18	19	21	40			
19	34	37	71	33	38	71
20	37	41	78			
21	9	12	21	37	40	77
22	10	10	20			
23	23	27	50	35	34	69
24	24	19	43			
25	47	48	95	46	48	94
26	49	50	99			
27	38	37	75	35	39	74
28	33	44	77			
29	31	34	65	34	31	65
30	31	41	72			
31	14	16	30	34	30	64
32	10	6	16			
33	22	15	37	33	36	69
34	23	21	44			
Total	841	891	1,732	611	653	1,264

Tables I and II give the incidence of caries for each groove in the molars of the two experimental groups as well as the incidence of bilateral symmetry, carious and noncarious, for each pair of grooves. It is worthy of note that there appears to be a trend toward greater incidence of caries and bilateral symmetry in the female for both groups, although, as shown later, this trend does not generally lead to significant differences in the individual groove comparisons.

A further observation which is to be made from Tables I and II is that caries incidence varies considerably from groove to groove, strongly indicating

that the grooves are far from equally susceptible to caries. For example, in Table I, the incidence of caries in the distal grooves of the maxillary third molars (No. 9 and No. 10, Fig. 1) is: left, 17 per cent, and right, 8 per cent. This is the low point. The high point of the range of incidence is in the central grooves of the mandibular second molars (No. 25 and No. 26, Fig. 1)

TABLE II

INCIDENCE OF CARIES BY GROOVES AND INCIDENCE OF SYMMETRY OF CARIES AND NONCARIES BY PAIRED GROOVES

Mixed Dietary Group (114 Males and 141 Females)

GROOVES	CARIES INCIDENCE			INCIDENCE OF SYMMETRY OF CARIES AND NONCARIES		
	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL
1	64	73	137	80	103	183
2	54	63	117			
3	80	102	182	71	108	179
4	79	83	162			
5	34	37	71	79	122	201
6	38	26	64			
7	69	85	154	86	106	192
8	79	80	159			
9	33	33	66	84	107	191
10	21	23	44			
11	38	59	97	81	92	173
12	45	58	103			
13	101	128	229	101	124	225
14	106	133	239			
15	79	108	187	95	115	210
16	84	112	196			
17	40	58	98	88	108	196
18	48	71	119			
19	76	106	182	84	108	192
20	76	113	189			
21	42	50	92	88	113	201
22	36	46	82			
23	75	81	156	82	101	183
24	65	79	144			
25	105	134	239	104	131	235
26	111	134	245			
27	85	106	191	90	110	200
28	85	109	194			
29	81	103	184	87	105	192
30	85	105	190			
31	36	46	82	84	96	180
32	32	49	81			
33	54	62	116	78	106	184
34	62	65	127			
Total	2,198	2,720	4,918	1,462	1,855	3,317

where we have: left, 95 per cent, and right, 99 per cent. We find a similar experience in Table II for the same grooves. This would seem to imply that incidence is related to position of the groove and that the different positions are related to the different susceptibilities of the grooves.

Statistical interpretation of these data clearly indicates the existence of a linear relationship between caries incidence in the grooves of the left and right quadrants for both the uniform and mixed dietary groups.* Student's "t"

*These tables and equations are available in the Dental Division, Naval Medical Research Institute, Bethesda, Md.

test for the difference between regression coefficients demonstrates that what differences do occur would happen on the average of more than 10 times in a 100 similar investigations. We can conclude, then, that the two groups are similar in so far as the linear relationship exhibited by them is concerned. Hence, they may be thrown together to determine a more stable regression equation from which it is possible to predict caries incidence for the grooves of the right quadrant, if we know the caries incidence for the left quadrant. A note of caution is necessary to avoid the reader's assuming that this equation will hold necessarily true for a given individual. That is to say, if we were to examine a series of individuals, making groove comparisons of caries incidence, we would find that for any of the individuals taken alone there would be considerable variation in symmetry. And if we were to attempt in any of these individuals to predict for the right side from the grooves of the left, we would be in error frequently. But if we were to sum up the caries experience for each groove over all the individuals, we would find a high degree of symmetry between left and right sides. Moreover, with a very large group the incidence of symmetry would approach very closely to the symmetry between groves characteristic of the population.

The correlation coefficients* computed for both groups separately and for the two groups thrown together range from 0.948 to 0.984, pointing to the existence of a high degree of association between the grooves of the quadrants. The coefficient of alienation† for the two groups together indicates that, on the hypothesis of bilateral symmetry, a prediction for the grooves of the right quadrant based upon certain knowledge of caries incidence for the grooves of the left quadrant would involve approximately 18 per cent guesswork.

The regression analysis and the determination of the degree of association herein discussed were made possible by the use of the technic of considering each groove individually, summing the caries experience for a particular groove over all the individuals of the group. The technic of summing the caries incidence of each side of the mouth without regard to the individual grooves, surfaces, or teeth for each individual studied does not allow for a method of measuring the degree of association of caries incidence which exists between the grooves, surfaces, or teeth of the quadrants.

Table III is a breakdown of the occurrence of bilateral symmetry for both caries and noncaries, as well as the occurrence of nonsymmetry. It is the base upon which Tables IV and V are constructed. These two sets of tables (IV and V) have three divisions, each constructed on a given assumption, as follows:

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*Coefficient of correlation

Let X = groove on left jaw molar
Y = corresponding groove on right jaw molar
N = total number of paired grooves
$\overline{X} = \Sigma X/N$ $\overline{Y} = \Sigma Y/N$

Then

$$\text{Coefficient of correlation} = r = \frac{\Sigma XY - N\overline{X}\overline{Y}}{\sqrt{[\Sigma X^2 - N\overline{X}^2]\ [\Sigma Y^2 - N\overline{Y}^2]}}$$

†Coefficient of alienation $= k = \sqrt{1 - r^2}$

A. Perfect symmetry of both caries and noncaries is assumed. The percentages given are the ratios of the incidence of bilateral symmetry, carious and noncarious, to the total numbers of pairs of grooves.

B. Perfect symmetry of caries is here assumed. The ratios are that of the incidence of bilateral symmetry of caries to the total number of pairs showing caries in at least one groove of the pair.

C. The assumption here made is that the maximum expected incidence of bilateral symmetry of caries for a given groove can be no greater than the lowest incidence of caries for one groove of the pair. That is, for example, Groove 1 has an incidence of 17 carious grooves, while its corresponding Groove 2 has an incidence of 22 carious grooves; obviously, no more than 17 of Grooves 1 and 2 can be bilaterally paired. But the actual number of

TABLE III

INCIDENCE OF CARIES AND NONCARIES IN BILATERALLY PAIRED GROOVES

GROOVE PAIRS	MALE			FEMALE		
	0,0	1,0	1,1	0,0	1,0	1,1
			Uniform diet			
1-2	20	21	9	18	16	16
3-4	9	21	20	15	16	19
5-6	37	9	4	35	11	4
7-8	15	20	15	19	10	21
9-10	39	10	1	41	5	4
11-12	28	15	7	31	10	9
13-14	1	5	44	0	2	48
15-16	10	12	28	6	9	35
17-18	25	13	12	22	14	14
19-20	6	17	27	5	12	33
21-22	34	13	3	34	10	6
23-24	19	15	16	19	16	15
25-26	0	4	46	0	2	48
27-28	7	15	28	4	11	35
29-30	11	16	23	3	19	28
31-32	30	16	4	29	20	1
33-34	19	17	14	25	14	11
Total	310	239	301	306	197	347
			Mixed diet			
1-2	38	34	42	54	38	49
3-4	13	43	58	32	33	76
5-6	60	35	19	100	19	22
7-8	21	28	65	41	35	65
9-10	72	30	12	96	34	11
11-12	57	33	24	58	49	34
13-14	4	13	97	2	17	122
15-16	23	19	72	18	26	97
17-18	57	26	31	60	33	48
19-20	23	30	61	15	33	93
21-22	62	26	26	79	28	34
23-24	28	32	54	41	40	60
25-26	1	10	103	2	10	129
27-28	17	24	73	18	31	92
29-30	18	27	69	19	36	86
31-32	65	30	19	71	45	25
33-34	38	36	40	60	35	46
Total	597	476	865	766	542	1,089

Code:

0,0 = no caries in either of the pair of grooves.
1,0 = caries in only one of the grooves of the pair.
1,1 = caries in both grooves of the pair.

Grooves 1 and 2 showing bilateral symmetry of caries is 9. Hence, we have the ratio 9/17 or 53 per cent.

Tables IV and V show that on assumption B we generally obtain the minimum of bilateral symmetry of caries incidence with a widespread range of relative symmetry for the paired grooves. On assumption C we obtain a maximum of bilateral symmetry of caries incidence with a slightly narrower range of relative symmetry. On assumption A we get values which tend to fall between those of assumptions B and C, and which have a much smaller range than those under the other assumptions.

TABLE IV

RATIOS BASED ON THREE DIFFERENT ASSUMPTIONS REGARDING SYMMETRY

GROOVE PAIRS	ASSUMPTION A		ASSUMPTION B		ASSUMPTION C		TOTALS		
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	ASSUMPTION A	ASSUMPTION B	ASSUMPTION C
Uniform diet									
1-2	0.580	0.680	0.300	0.500	0.529	0.696	0.630	0.403	0.625
3-4	0.580	0.680	0.488	0.500	0.690	0.792	0.630	0.513	0.736
5-6	0.820	0.780	0.308	0.267	0.500	0.667	0.800	0.286	0.571
7-8	0.600	0.800	0.429	0.677	0.714	0.808	0.700	0.545	0.766
9-10	0.800	0.900	0.091	0.444	0.333	0.800	0.850	0.250	0.625
11-12	0.700	0.800	0.318	0.474	0.583	0.692	0.750	0.390	0.640
13-14	0.900	0.960	0.898	0.960	0.978	1.000	0.930	0.929	0.989
15-16	0.760	0.820	0.700	0.795	0.848	0.897	0.790	0.750	0.875
17-18	0.740	0.720	0.480	0.500	0.667	0.667	0.730	0.491	0.667
19-20	0.660	0.760	0.614	0.733	0.794	0.892	0.710	0.674	0.845
21-22	0.740	0.800	0.187	0.375	0.333	0.600	0.770	0.281	0.474
23-24	0.700	0.680	0.516	0.484	0.696	0.789	0.690	0.500	0.738
25-26	0.920	0.960	0.920	0.960	0.979	1.000	0.940	0.940	0.989
27-28	0.700	0.780	0.651	0.761	0.848	0.946	0.740	0.708	0.900
29-30	0.680	0.620	0.590	0.596	0.742	0.823	0.650	0.593	0.785
31-32	0.680	0.600	0.200	0.048	0.400	0.167	0.640	0.122	0.313
33-34	0.660	0.720	0.452	0.440	0.636	0.733	0.690	0.446	0.676
All groove pairs	0.719	0.768	0.557	0.638	0.762	0.844	0.743	0.598	0.804
Mixed diet									
1-2	0.702	0.730	0.553	0.563	0.778	0.778	0.718	0.558	0.778
3-4	0.623	0.766	0.574	0.697	0.734	0.916	0.702	0.638	0.827
5-6	0.693	0.865	0.352	0.537	0.559	0.846	0.788	0.432	0.683
7-8	0.754	0.752	0.699	0.650	0.942	0.813	0.753	0.674	0.872
9-10	0.737	0.759	0.286	0.244	0.571	0.478	0.749	0.264	0.523
11-12	0.710	0.652	0.421	0.410	0.632	0.586	0.678	0.414	0.604
13-14	0.886	0.879	0.882	0.878	0.960	0.953	0.882	0.879	0.956
15-16	0.833	0.816	0.791	0.789	0.911	0.898	0.823	0.790	0.904
17-18	0.772	0.766	0.544	0.593	0.775	0.828	0.769	0.572	0.806
19-20	0.737	0.766	0.670	0.738	0.802	0.877	0.753	0.710	0.846
21-22	0.772	0.801	0.500	0.548	0.722	0.739	0.788	0.526	0.732
23-24	0.719	0.716	0.628	0.600	0.831	0.759	0.718	0.613	0.792
25-26	0.912	0.929	0.911	0.928	0.981	0.963	0.922	0.921	0.971
27-28	0.789	0.780	0.753	0.748	0.859	0.868	0.784	0.750	0.864
29-30	0.763	0.745	0.719	0.705	0.852	0.835	0.753	0.711	0.842
31-32	0.737	0.681	0.388	0.357	0.594	0.543	0.706	0.370	0.564
33-34	0.684	0.752	0.526	0.568	0.741	0.742	0.722	0.548	0.741
All grove pairs	0.754	0.774	0.645	0.668	0.825	0.832	0.765	0.657	0.829

A = Assumption of perfect symmetry of total carious and noncarious groove pairs.
B = Assumption of perfect symmetry of total carious groove pairs only. Noncarious groove pairs are not considered.
C = Assumption that maximum expected incidence of bilateral symmetry of caries for a given groove can be no greater than the lowest incidence of caries for one groove of the pair.

We used the data on assumption A. Our reason for so doing being that, generally, symmetry as between the grooves involves symmetry of caries and noncaries, for when we predict a given caries incidence for a given groove from knowledge of the incidence for the other groove of the pair, we are to some degree predicting also the noncaries incidence. Furthermore, the data on bilateral symmetry for both caries and noncaries appear to fall between two values, which we choose to consider as of the nature of upper and lower limits of symmetry.

TABLE V

RATIOS BASED ON THREE DIFFERENT ASSUMPTIONS REGARDING SYMMETRY

Uniform and Mixed Diets Together

	ASSUMPTION A		ASSUMPTION B		ASSUMPTION C		TOTALS		
GROOVE PAIRS	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	ASSUMPTION A	ASSUMPTION B	ASSUMPTION C
1-2	0.665	0.721	0.481	0.546	0.671	0.739	0.693	0.516	0.707
3-4	0.610	0.747	0.549	0.660	0.722	0.888	0.682	0.605	0.805
5-6	0.732	0.847	0.343	0.464	0.548	0.812	0.791	0.398	0.662
7-8	0.707	0.768	0.625	0.656	0.816	0.811	0.738	0.641	0.814
9-10	0.756	0.800	0.248	0.278	0.542	0.536	0.777	0.262	0.538
11-12	0.707	0.695	0.397	0.422	0.620	0.597	0.699	0.409	0.607
13-14	0.890	0.905	0.887	0.899	0.966	0.966	0.896	0.894	0.966
15-16	0.811	0.821	0.763	0.790	0.877	0.892	0.814	0.778	0.885
17-18	0.762	0.758	0.524	0.569	0.741	0.785	0.758	0.550	0.766
19-20	0.713	0.768	0.652	0.737	0.800	0.881	0.741	0.699	0.846
21-22	0.762	0.805	0.426	0.513	0.630	0.714	0.783	0.473	0.676
23-24	0.713	0.710	0.598	0.572	0.786	0.765	0.710	0.585	0.775
25-26	0.915	0.942	0.914	0.936	0.980	0.940	0.927	0.926	0.976
27-28	0.762	0.784	0.721	0.751	0.856	0.888	0.772	0.738	0.874
29-30	0.738	0.716	0.681	0.675	0.821	0.832	0.724	0.678	0.827
31-32	0.719	0.663	0.333	0.286	0.548	0.473	0.687	0.306	0.505
33-34	0.677	0.747	0.505	0.538	0.710	0.740	0.713	0.521	0.725
All groove pairs	0.743	0.772	0.620	0.660	0.798	0.831	0.759	0.641	0.816

The percentages of bilateral symmetry are compared within the experimental groups and between them. The critical ratio (C. R.) obtained in the comparisons is a normally distributed variable whose values may be obtained from a table of normal areas. The hypothesis in each comparison is that the two percentages do not differ. The value, C.R. = 2.00, is chosen as the critical value and has a probability of P = 0.0455. That is, a value as great or greater than C.R. = 2.00 would occur by chance on the average of about 5 times in a hundred comparisons.* Thus we have compared the following:

a. Males----------uniform vs. mixed diet groups
b. Females----------uniform vs. mixed diet groups

*Formula used for determining critical ratio (C.R.) applied to data in Tables IV and V.

Pu = percentage of symmetrical occurrence of caries and noncaries in Nu pair of grooves in uniform dietary groups.

Pm = percentage of symmetrical occurrence of caries and noncaries in Nm corresponding pair of grooves in mixed dietary group.

Po = percentage of symmetrical occurrence of caries and noncaries when grooves for both uniform and mixed dietary groups taken together.

$Q_o = 1\text{-}P_o$

$$\sigma Pu - Pm = \sqrt{\frac{P_o\,Q_o}{Nu} + \frac{P_o\,Q_o}{Nm}} \qquad \text{Then C.R.} = \frac{Pu - Pm}{\sigma Pu - Pm}$$

c. Uniform diet----------males vs. females
d. Mixed diet----------males vs. females
e. Uniform vs. mixed dietary groups.

A value of C.R. = 2.00 or greater occurred only once in paired groove comparison of Groups a, b, c, and e and twice in group d. This did not occur in the same pair of grooves in every instance and each significant difference occurred in the grooves of the maxillary quadrants. Generally, then, we may consider the two groups as similar and throw them together to obtain rates characteristic of the entire group.*

It is now possible to return to Table V and to compare our findings for the total of both mixed and uniform dietary groups. The general observations made before hold true. We see that the average percentage of bilateral symmetry ranges from 64.1 per cent on assumption B to 81.6 per cent on assumption C with the average on assumption A, 75.9 per cent, falling between them. The range of relative frequency of bilateral symmetry on assumption C is from approximately 50 per cent to 97 per cent; on assumption B from 26 per cent to 92 per cent; and on assumption A from 68 per cent to 92 per cent. It is apparent that regardless of what assumptions we make the degree of symmetry is appreciable, but that, at the same time, there is a material degree of asymmetry.

TABLE VI

COMPARISON OF QUADRANTS

GROUPS	QUADRANTS				C.R.
	Maxillary left vs. maxillary right				
Uniform diet	Males	0.676	Females	0.768	2.30
Mixed diet	Males	0.702	Females	0.774	2.88
Males	Uniform	0.676	Mixed	0.702	0.76
Females	Uniform	0.768	Mixed	0.774	0.19
Diets	Uniform	0.722	Mixed	0.742	0.87
Uniform + mixed	Males	0.694	Females	0.773	3.43
	Mandibular left vs. mandibular right				
Uniform diet	Males	0.737	Females	0.768	1.24
Mixed diet	Males	0.776	Females	0.774	0.13
Males	Uniform	0.737	Mixed	0.776	1.86
Females	Uniform	0.768	Mixed	0.774	0.30
Diets	Uniform	0.752	Mixed	0.775	1.64
Uniform + mixed	Males	0.764	Females	0.772	0.57

It was noted previously that there seemed to be a general trend toward greater incidence of caries and bilateral symmetry in the female. The comparisons of individual grooves or pairs of grooves for the sexes does not indicate that the trend makes for significant differences. However, when we compare the maxillary quadrants of the sexes, it is observed (Table VI) that males and females differ significantly within the dietary groups as well as when these groups are thrown together. This does not apply, however, to the comparison of the occurrence of bilateral symmetry in the mandibular quad-

*Detailed tables of these data are available in Dental Division, Naval Medical Research Institute, Bethesda, Md.

rants of the sexes. When the two diets and the maxillary and mandibular quadrants are lumped together, and the sexes compared, we get a value C.R. = 2.64, indicating that the males and females differ significantly in the relative incidence of symmetry of caries and noncaries.*

The methods we have used, when considered all together, emphasize strongly the fact that mere summation of caries incidence on both sides and then comparing on the assumption of perfect symmetry leads to discrepancies which may affect any conclusions drawn from such data. It has been shown that, on any assumption, the grooves differ between themselves as to the frequency of caries attack. If then, in a study, we have teeth missing, and these missing teeth are not bilaterally paired, our sum of carious teeth on either side may show more than chance differences when compared, and lead to conclusions of real differences between the quadrants.

TABLE VII

CARIES INCIDENCE UNIFORM DIET

GROOVES	MALE		FEMALE		TOTAL	
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT
Maxilla						
1-2	17	22	23	25	40	47
3-4	32	29	30	24	62	53
5-6	8	9	13	6	21	15
7-8	29	21	26	26	55	47
9-10	9	3	8	5	17	8
Total	95	84	100	86	195	170
Mandible						
11-12	12	17	13	15	25	32
13-14	45	48	48	50	93	98
15-16	35	33	40	39	75	72
17-18	18	19	21	21	39	40
19-20	34	37	37	41	71	78
21-22	9	10	12	10	21	20
23-24	23	24	27	19	50	43
25-26	47	49	48	50	95	99
27-28	38	33	37	44	75	77
29-30	31	31	34	41	65	72
31-32	14	10	16	6	30	16
33-34	22	23	15	21	37	44
Total	328	334	348	357	676	691
Total	423	418	448	443	871	861

Further more, the mere summation of quadrants appears to mask a fact which may have some significance in the case where teeth may be missing. Upon observation of data in Tables VII and VIII, we note that in every case the left quadrant in the maxillae shows a higher caries incidence, while in the mandibular quadrants, the right shows the higher incidence; but when maxillary and mandibular quadrants are summed, we find the totals very nearly the same.

Hence, we can conclude that our data strongly indicate that both position and structure play a role in the caries-producing mechanism and that mere

*These values are not seen in the tables here presented.

summation for the quadrants provides neither a measure of the degree of association, nor a means of determining the relative susceptibility of the grooves, surfaces, or teeth when bilateral symmetry is considered.

TABLE VIII

CARIES INCIDENCE MIXED DIET

GROOVES	MALE		FEMALE		TOTAL	
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT
Maxilla						
1-2	64	54	73	63	137	117
3-4	80	79	102	83	182	162
5-6	34	38	37	26	71	64
7-8	69	79	85	80	154	159
9-10	33	21	33	23	66	44
Total	280	271	330	275	610	546
Mandible						
11-12	38	45	59	58	97	103
13-14	101	106	128	133	229	239
15-16	79	84	108	112	187	196
17-18	40	48	58	71	98	119
19-20	76	76	106	113	182	189
21-22	42	36	50	46	92	82
23-24	75	65	81	79	156	144
25-26	105	111	134	134	239	245
27-28	85	85	106	109	191	194
29-30	81	85	103	105	184	190
31-32	36	32	46	49	82	81
33-34	54	62	62	65	116	127
Total	812	835	1,041	1,074	1,853	1,909
Total	1,092	1,106	1,371	1,349	2,463	2,455
Uniform and Mixed Together Diets						
Maxilla	375	355	430	361	805	716
Mandible	1,140	1,169	1,389	1,431	2,529	2,600
Total	1,515	1,524	1,819	1,792	3,334	3,316

SUMMARY

1. When the caries experience for each groove is sumed up over all the individuals of a group, a high degree of association has been found between the bilaterally paired grooves of the molar teeth of the white rats in this study. In the individual case, however, the degree of bilateral symmetry is subject to great variation.

2. In approximately 75 per cent of the cases, the condition of caries or noncaries in one of the bilaterally paired grooves will be duplicated in the other member of the pair.

3. The average incidence of caries varies from groove to groove and appears to be related to groove position.

4. An appreciable degree of asymmetry exists which would materially affect any conclusions of experiments based upon the hypothesis of perfect symmetry.

5. A method has been demonstrated by means of which it is possible to determine the degree of symmetry present between bilaterally paired grooves.

6. Incidental findings show sex differences existing in caries incidence. Bilateral quadrant differences between the maxilla and mandible were obscured by summation of total caries incidence without regard to groove.

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AN EVALUATION OF VARIOUS TECHNICS FOR REDUCING DECALCIFICATION OF ENAMEL

I. In Extracted, Erupted, Noncarious Third Molar Teeth

LT. COLONEL HUBERT B. PALMER, USAF (DC), SUSIE OVERSTREET, B.S., AND MARILYN SACKS, B.S.

Department of Dental Research, Dental Division, Army Medical Research and Graduate School, Army Medical Center, Washington, D. C.

INTRODUCTION

CHEYNE,[1] Bibby,[2] Knutson and Armstrong,[3, 4, 5] Knutson, Armstrong, and Feldman,[6] Jordan, Wood, Allison, and Irwin,[7] Crawford,[8] Younger,[9] and Gillard, Boelsche, and Summers[10] have reported a decrease in the incidence of dental caries in groups of patients following the employment of a method utilizing either a certain fluoride solution or other pharmaceuticals in combination.

Volker,[11] Bibby,[12] Muhler and Van Huysen,[13] and Phillips[14] have shown with either powdered enamel or enamel fragments that both were less soluble to acids following their exposure to a fluoride solution. Phillips[14] has shown that the hardness and impermeability of enamel was increased following exposure to a fluoride solution. Bowes and Murray,[15] French, Welch, Simmons, Le Fevre, and Hodge,[16] Le Fevre and Hodge,[17] Armstrong and Brekhus[18] in their studies of either enamel or dentin have shown variations in the chemical composition of teeth. Karlstron,[19] Berghash and Hodge,[20] Hodge and McKay,[21] Van Huysen,[22, 23] Van Huysen, Hodge, and Warren,[24] Scott and Wyckoff[25] have shown in studies which concerned either density, hardness, radiopacity, or photomicrography of enamel and dentin that the physical properties of these tissues vary within the respective tisues of each tooth.

Clinical studies performed by Knutson, Klein, and Palmer[26] have shown that teeth vary in their susceptibility to dental caries. Similar studies by Palmer, Nodich, and Kaeyon,[27] and Schlack and Birren[28] have shown that the dental caries incidence of individuals of same age groups vary widely, depending upon their place of nativity and residence during childhood. Klinkenberg and Bibby[29] have reported a decrease in the incidence of dental caries for groups of individuals between 18 and 40 years of age whose teeth had been treated with solutions of 1.0 per cent sodium fluoride and 0.06 per cent lead fluoride.

Since teeth vary widely in chemical and physical properties, only extracted, erupted, noncarious third molar teeth of human beings have been utilized in this experiment. The purpose of this investigation has been to determine which of the technics—namely, (a) a modification of Knutson's method[30] utilizing chemically pure 2 per cent sodium fluoride solution, (b) a method utilizing an acidulated fluoride solution* or (c) Gottlieb's method[31]

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*The acidulated fluoride solution having a pH 4.67 is composed of 0.26 molar of chemically pure sodium fluoride solution and 1.50 per cent acetic acid solution. This was applied to the teeth in a manner similar to that described for 2 per cent sodium fluoride solution.

utilizing Zin-Ferro-Nitrate* solutions—to be the most suitable for controlling decalcification† in enamel caused by growth products of an oral strain of the *Lactobacillus acidophilus.*

METHOD OF STUDY

This in vitro study which eliminates many human variations has been found upon the extensive work of McIntosh, James, and Lazarus-Barlow,[32, 33] Rodriguez,[34] Bunting and Palmerlee,[35] Bunting, Nickerson, Hail and Crowley,[36] Hadley, Bunting, and Delves,[37] Jay, Hadley, and Bunting,[38] and Palmer, Overstreet, and Sacks[39] concerning the *Lactobacillus acidophilus.* Preliminary studies by Palmer, Overstreet, and Sacks[39] indicate that it may not be necessary for proteolysis to precede decalcification in the destruction of intact enamel, dentin, and cementum.

Extracted, erupted, noncarious whole third molar teeth, representing 148 adults, were thoroughly cleaned with scalers, rubber cups, and pumice. They were then dried at 42° C. for 24 hours after which the teeth were weighed and divided into 4 groups. In Groups 1, 2, and 3, one-half the crown and the entire root portion of each tooth were first covered with paraffin. In Group 1 the exposed one-half of each crown of the 19 teeth was covered with absorbent cotton saturated with acidulated fluoride solution. The cotton was kept moist and allowed to remain upon the tooth for 35 minutes. The cotton was removed and the tooth allowed to dry for 5 minutes. In Group 2 the exposed one-half of each crown of the 18 teeth was similarly treated with chemically pure 2 per cent fluoride solution. In Group 3 the exposed one-half of each crown of 29 teeth was treated with Zin-Ferro-Nitrate solutions. Applying the solutions with cotton applicators the exposed portion was thoroughly washed with Zin-Ferro Solution No. 1 for 1 minute and then allowed to dry. Zin-Ferro Solution No. 2 was then applied to the crown and permitted to dry for 90 seconds. Zin-Ferro Solution No. 3 was then applied and permitted to remain until the entire surface was covered with a white precipitate. Ten per cent silver nitrate solution was next applied and allowed to remain for 5 minutes. Surplus precipitate was then removed, the crown dried, and the process of re-applying Zin-Ferro Solutions 2 and 3 and the 10 per cent silver nitrate solutions was repeated twice. Following the treatment of all the teeth in Groups 1, 2, and 3 the paraffin covering on each tooth was removed.

Teeth in Group 4 were divided into 4 subgroups. Group 4a was composed of 25 upper right third molar teeth. Group 4b included 24 upper left third molar teeth. Group 4c was composed of 23 lower right third molar teeth. Group 4d included 19 lower left third molar teeth. All of the teeth in the 4 subgroups served as controls, none being treated. With the aid of stainless steel wire, each tooth in the experimental and control groups was placed in an

*Zin-Ferro-Nitrate as described by the investigators was used to designate four solutions employed in this study. These solutions were: (a) chemically pure benzine, (b) 40 per cent zinc chloride solution with a wetting agent, (c) 20 per cent potassium ferrocyanide solution with a wetting agent, and (d) 10 per cent silver nitrate solution. The wetting agent in each case was a 1 per cent solution of polyoxyalkalene sorbitan monolaurate. Test solutions (a), (b), and (c) above were prepared by the Yarbrough Drug Company of Dallas, Texas, under the trade name "Zin-Ferro Solutions Nos. 1, 2, and 3."

†Decalcification of dental tissues as referred to in this report is based upon the difference noted between the original and final weights of the tooth following exposure to the *Lactobacillus acidophilus.*

individual test tube containing 10 c.c. of 70 per cent ethyl alcohol for 20 minutes. (Control studies following the alcohol treatment showed no growth.) All teeth were then placed in individual test tubes and immersed under 10 c.c. of glucose-serum broth,* pH 7.0, seeded with 0.5 of 1 c.c. of 48-hour broth culture of *Lactobacillus acidophilus* oral strain 12B. Incubation at 37° C. for 30 days followed. Teeth were transferred to new broth and reinoculated on Monday, Wednesday, and Friday of each week. At weekly intervals hydrogen-ion determinations of the 48-hour broth cultures were made with the Beckman pH meter. Following the 30-day incubation period, all the teeth were placed in alcohol for 20 minutes, washed clean, and then dried at 42° C. for 24 hours after which they were weighed.

A COMPARATIVE STUDY OF VARIOUS AGENTS FOR REDUCING ENAMEL DECALCIFICATION. WHOLE TEETH WERE CONTINUOUSLY EXPOSED TO LACTOBACILLUS ACIDOPHILUS ORAL STRAIN 12B FOR THIRTY DAYS.

GROUP NO.	TYPE OF TREATMENT	NO. TEETH	TOTAL WEIGHT OF GROUPS IN GRAMS		% WT. LOSS	% REDUCTION IN DECALCIFICATION
			ORIGINAL	FINAL		
1	ACIDULATED FLUORIDE SOLUTION	19	33.9286	31.3795	7.52	32.38
2	2% SODIUM FLUORIDE SOLUTION	18	31.3775	29.0279	7.49	32.65
3	ZIN-FERRO-NITRATE SOLUTION	29	51.4137	46.3705	9.81	11.79
4	CONTROLS	93	165.0469	146.6972	11.12	——

Table I.

RESULTS

Table I shows that teeth treated with an acidulated fluoride solution had a mean weight loss of 7.52 per cent following a 30-day exposure to *Lactobacillus acidophilus* oral strain 12B. Teeth treated with a 2 per cent sodium fluoride solution showed a mean weight loss of 7.49 per cent. Teeth treated with the Zin-Ferro-Nitrate solutions showed a mean weight loss of 9.81 per cent. The control teeth showed a mean weight loss of 11.12 per cent. Teeth treated with the acidulated fluoride solution showed a mean reduction in decalcification of 32.38 per cent while those treated with the 2 per cent sodium fluoride solution showed a mean reduction in decalcification of 32.65 per cent. Those teeth treated with the Zin-Ferro-Nitrate solutions showed only 11.79 per cent reduction in decalcification.

Table II indicated that untreated teeth among individuals demonstrate a wide range in resistance to decalcification. Tooth 16 of individual 972AB was 447.98 per cent more susceptible to decalcification than was Tooth 1 of individual 1169BC. Teeth 1 and 16 from individual 1211AE showed little

*The glucose-serum broth was composed of veal infusion broth with 1 per cent glucose and 10 per cent horse serum.

difference in susceptibility to decalcification. However, Teeth 1 and 32 from individual 1169BC demonstrated a difference in susceptibility to decalcification of 160.40 per cent.

Defective areas, including fracture lines in enamel, readily adsorbed the 10 per cent silver nitrate solution employed in the Zin-Ferro-Nitrate study. The black disfiguring stain, indicative of silver nitrate adsorption, of the enamel could not be removed with the aid of either rubber cups and pumice or scalers. Enamel having no defects showed no such stain following the use of 10 per cent silver nitrate solution.

A COMPARATIVE STUDY OF THE RESISTANCE TO DECALCIFICATION OF EXTRACTED, ERUPTED, NON-CARIOUS UNTREATED MOLAR TEETH FROM THE SAME INDIVIDUAL.

INDIVIDUAL'S HISTORY				WEIGHT IN GRAMS OF WHOLE TOOTH BEFORE AND AFTER EXPOSURE TO LACTOBACILLUS ACIDOPHILUS		
SERIAL NO	AGE	SEX	TOOTH NO.	ORIGINAL WEIGHT	FINAL WEIGHT	% WT. LOSS.
1169BC	47	M	1	1.6328	1.5842	2.98
"	"	"	32	1.9576	1.8019	7.76
1211 AE	45	F	1	1.7839	1.6370	8.24
"	"	"	16	1.7251	1.5801	8.41
1200 AB	32	M	1	1.1679	1.0292	11.88
"	"	"	16	2.1503	1.8871	12.25
1263 AB	23	F	32	1.3563	1.2090	10.89
"	"	"	17	1.4464	1.2639	12.62
972 AB	23	F	17	2.0169	1.8821	6.69
"	"	"	16	1.1216	0.9385	16.33
1260 ABC	30	M	32	1.6642	1.5337	7.85
"	"	"	17	1.6962	1.5589	8.10
"	"	"	1	1.6255	1.4752	9.25

Table II.

HYDROGEN-ION DETERMINATION OF BROTH CULTURES

The mean pH for the 48-hour broth cultures for each of the 4 groups showed no significant differences. Both cultures at the beginning of the incubation period were near pH 7.0. After 19 hours' incubation at 37° C. broth cultures demonstrated at pH near 4.20. Depending upon whether the teeth were transferred to new broth at the end of 48 or 72 hours, teeth remained in broth cultures having a pH near 4.20 for 29 or 53 hours before they were exposed to an environment of pH 7.0 again.

DISCUSSION

It was also observed that the effect of either an acidulated fluoride solution, a 2 per cent sodium fluoride solution, or a Zin-Ferro-Nitrate solution for controlling decalcification varied widely with individual teeth (Table III). Definite evidence presented showed that extracted, untreated, erupted non-

carious third molar teeth from the same individual and those from other individuals vary widely in their resistance to the growth products of the *L. acidophilus* (Table II).

In this in vitro study many human variations common to clinical studies on dental caries have been eliminated: (a) individual differences in the number of lactobacilli present in the saliva, (b) differences in the species of lactobacilli present—some species being capable of producing more acid than others and hence being capable of producing a lower pH, (c) differences in the practice of oral hygiene and plaque removal by patients, (d) differences in the buffer capacities of various salivas, (e) the presence or absence of antibodies in the saliva for the *L. acidophilus*, (f) differences in the individual diets, and (g) variations in the physiologic assimilation of foods. Such biologic variations as well as variations in tooth resistance among indivduals tend to mask the true significance of a testing agent when studied clinically.

A COMPARATIVE STUDY OF EXTRACTED, ERUPTED THIRD MOLAR TEETH: FOLLOWING THE APPLICATION OF AN ACIDULATED FLUORIDE SOLUTION TO HALF THE CROWN OF EACH TOOTH FOR THIRTY-FIVE MINUTES, THE WHOLE TOOTH WAS CONTINUOUSLY EXPOSED TO LACTOBACILLUS ACIDOPHILUS ORAL STRAIN 12B FOR THIRTY DAYS

INDIVIDUAL'S HISTORY						WEIGHT IN GRAMS OF THE WHOLE TOOTH BEFORE AND AFTER EXPOSURE TO LACTOBACILLUS ACIDOPHILUS		
SERIAL	PLACE OF BIRTH	PLACE OF RESIDENCE AT TIME OF EXTRACTION	SEX	AGE	TOOTH	ORIGINAL WEIGHT	FINAL WEIGHT	% WT. LOSS
941	HUBBARD ,TEXAS	SAME	F	37	16	1.5860	1.4624	7.80
916	LONG ISLAND, N.Y.	SAME	F	18	16	1.5962	1.3840	13.30
1089A	McKINNEY, TEXAS	SAME	M	35	16	2.3672	2.2539	4.79
1095	HANNIBAL, MO.	SAME	F	25	16	1.3631	1.2228	10.30
1111	DANSVILLE, N.Y.	SAME	M	53	16	1.8549	1.7092	7.86
1012A	ARKANSAS	SAME	F	48	1	1.7510	1.6765	4.26
826B	CHARLEROI, PENN.	SAME	M	21	1	1.3692	1.2679	7.40
911	NORTH PLATT, NEBRASKA	OMAHA, NEBRASKA	F	20	1	1.9097	1.7818	6.70
1045B	KENTUCKY	SAME	M	22	1	1.4382	1.2962	9.88
1022	PARIS, TEXAS	SAME	M	29	1	1.8068	1.6770	7.19
965	MANSFIELD, TEXAS	SAME	M	33	32	2.0180	1.9061	5.55
842	MURPHY, N.C.	SAME	M	26	32	2.4921	2.3941	3.94
1036	POST, TEXAS	SAME	F	17	32	1.4113	1.2603	10.70
973	CALIFORNIA	SAME	M	20	17	2.0664	1.9909	3.66
866B	GEORGIA	SAME	F	19	17	1.7316	1.5988	7.67
922	RECKTOR, ARKANSAS	SAME	M	19	17	1.9582	1.7581	10.22
1032	PORTALIS, NEW MEXICO	SPEN, TEXAS	M	43	17	1.7838	1.6559	7.18
1038	LUBBOCK CO., TEXAS	SAME	F	28	17	1.5790	1.4467	8.38
1123	LONG ISLAND, N.Y.		F	19	17	1.8459	1.6369	11.33
					TOTAL	33.9286	31.3795	
GROUP NO. 1					PERCENTAGE MEAN WEIGHT LOSS			7.52

Table III.

This in vitro study has a clinical application in that bacteriologic methods were used which permit the exposure of teeth to an environment having a pH above and below the isoelectric point of enamel during each period of incubation. In the decalcification of teeth leading to the development of dental caries in the oral cavity, the area attached is exposed intermittently to either an environment (the dental plaque) having a pH below the isoelectric point

of enamel or to a medium (the saliva) having a pH above the isoelectric point of enamel. The time element in this experiment has been of sufficient duration for demonstrating variations in resistance of individual teeth to decalcification and for evaluating the effects that testing solutions have upon teeth when exposed to an environment conducive to decalcification.

That the acidulated fluoride solution as applied in this experiment was not more effective for controlling decalcification of enamel than was the 2 per cent sodium fluoride solution may be attributed to the fact that the first solution was only a 1 per cent solution of sodium fluoride.

The technic of applying Zin-Ferro-Nitrate solutions was most cumbersome and time-consuming and least effective. In as much as there does exist among teeth a wide variation in resistance to decalcification, the effect of the solutions tested in this investigation are now being studied and evaluated upon matched enamel-dentin fragments from the same tooth.

One questions the importance of tooth resistance to decalcification and the applications of solutions to the teeth to decrease decalcification when there normally exists a wide variation in resistance to decalcification among noncarious molar teeth which have been extracted from the same individual.

CONCLUSIONS

1. The technics for reducing decalcification of enamel in vitro utilizing an acidulated fluoride solution or 2 per cent sodium fluoride solution were 64 per cent more effective than Gottlieb's method, utilizing the Zin-Ferro-Nitrate solutions.

2. The effect of either an acidulated fluoride solution, a 2 per cent sodium fluoride solution, or the Zin-Ferro-Nitrate solution for reducing decalcification in vitro varied with individual teeth.

3. Two or more extracted, erupted, untreated noncarious third molar teeth from the same individual exhibited differences in resistance to decalcification. Likewise, among individuals, untreated teeth showed wide variation in tooth resistance and susceptibility to decalcification.

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THE ISOLATION, PROPAGATION, AND ACTIVITY OF A VIRAL AGENT ASSOCIATED WITH DENTINAL DESTRUCTION

NAOMI C. TURNER, ED.M., GEORGE E. CROWELL, D.M.D., AND HOWARD DEXTER, D.D.S.

From the Bio-Chemistry Laboratory of the Forsyth Dental Infirmary for Children, Boston, Mass.

SINCE Miller's finding of tooth destruction in the presence of human saliva and bread pabulum, a great deal of attention has been given to the possible role of bacteria in the etiology of dental caries. Less attention has been given to the possibility that a viral agent may be involved in the destruction of the tooth.

Pincus[1] gave consideration to the possibility that a bacteriophage was operative in the saliva. Enders[2] reported various viral agents which may reside in saliva. Aisenberg and Grubb[3] showed the pulp chamber as a possible portal of entry for the poliomyelitis virus in the rhesus monkey.

In this paper we report the isolation of a viral agent from fresh dentinal decay, the propagation of this agent in the developing chick embryo, and its activity on the rat's incisor.

METHOD

The surface layer of a decayed area of a tooth in the patient's mouth or of an extracted tooth immediately following extraction is first removed with a spoon curette. The underlying fresh dentinal decay is then spooned out and placed in a sterile mortar. It is ground with a sterile pestle with or without washed preheated sand. To this material a small amount of physiologic saline is added, and grinding is continued. When the material is pasty in consistency, 5 ml. of physiologic saline is added for approximately each 0.5 ml. of the spooned-out dentinal decay. This suspension is placed in a Seitz filter (0.2-0.3 μ) and drawn through with suction into a receiving side-arm Erlenmeyer flask using sterile precautions.

The filtrate is then drawn into a sterile hypodermic syringe and 0.5 ml. of this filtrate is injected onto the chorio-allantoic membrane of a developing chick embryo. Ages of the embryos used during the first weeks of the study varied from 7 to 12 days; 8- to 10-day embryos became the age of choice.

In preparing the egg for inoculation the method of Burnet,[4] Goodpasture,[5] and others was followed. The egg was candled; an opening was made over the embryo with a dental diamond stone. The diamond stone cuts quickly at high speed with little heat. We have encountered little injury to the underlying membrane and a minimum of hemorrhage. The air-sac end of the egg was opened[6] to allow the embryo to fall for easier manipulation.

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After the injection of 0.5 ml. of dentinal decay filtrate, the opening in the egg was sealed with dental wax and the egg returned to the incubator. Eggs were opened after intervals of 24 to 120 hours. Forty-eight hours was selected as the preferred incubation period. The chorio-allantoic membrane was found in better condition at this age than later for the preparation of impression slides. Allantoic fluid was, at this stage, easily withdrawn. To store the embryo in a refrigerator for chilling[7] prior to withdrawal of allantoic fluid made the withdrawal easier and seemed to prevent admixture with blood.

The technic of transfer was to insert a sterile hypodermic syringe into the chorio-allantoic sac after opening the egg according to standardized methods. Available allantoic fluid was withdrawn. Then 0.4 ml. of this fluid was injected into the next egg of the serial passage. Any excess allantoic fluid was refrigerated for later use.

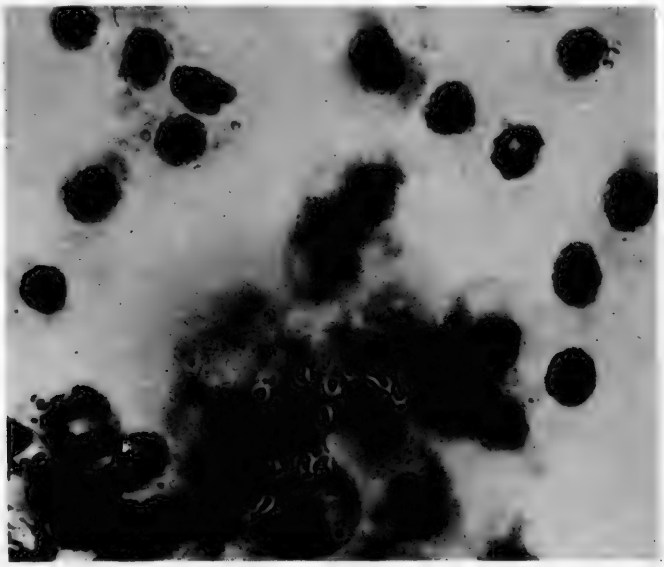

Fig. 1.—Photomicrograph of cells from smear of chorio-allantoic membrane of an 11-day embryo which was part of the serial passage starting with dentinal decay filtrate. Note cytoplasmic inclusion bodies stained with Seller's stain. (×2400)

The impression slides from the chorio-allantoic membrane of the injected developing embryo were prepared and stained for 5 seconds in Seller's stain[8] and examined for cytoplasmic inclusion bodies. Such bodies have been seen from the fifth to the twenty-fifth passage. The number of cells showing inclusion bodies and the number of inclusion bodies per cell in our R series increased with passage up to the seventeenth. In our present limited experience, the fresher the dentinal decay, the earlier in passage do the inclusion bodies appear. The form of inclusion body found is illustrated in Figs. 1 and 2.

The stored allantoic fluid saved from eggs whose chorio-allantoic membrane showed inclusion bodies is inoculated onto nutrient agar and into thioglycollate

media to test for freedom from or contamination with bacteria. After 48 hours in the incubator, the agar and thioglycollate media are read.

In addition to testing for freedom from bacteria, the pH of the allantoic fluid was taken. The pHs have shown remarkable consistency running thus far from pH 6.4 to pH 7.5. Positive TPA* values were obtained when this allantoic fluid was tested by the Atlas and Hottle technic.[9]

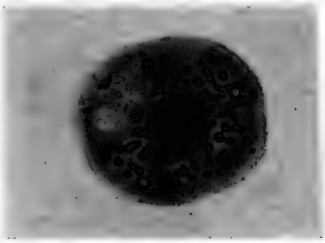

Fig. 2.—Photomicrograph of single cell showing cytoplasmic inclusion bodies from smear of chorio-allantoic membrane of 11-day embryo, seventh in serial passage starting with pooled dentinal decay filtrate.

Fig. 3.—Photomicrograph of the body of a rat's incisor immersed in same control fluid as in Fig. 4. (×80)

We sought to test possible activity on tooth structures of a viral agent contained in the allantoic fluid of the injected and passaged embryos. A rat's incisors were extracted using surgical forceps. These 2 teeth were dropped into Zephiran Chloride (0.1 ml. concentrated Zephiran Chloride, 12.8 ml. distilled

*Tryptophane-perchloric acid.

water) for a period of 2 hours. One of these 2 teeth, upon removal from Zephiran Chloride, was dropped into bacteria-free allantoic fluid, collected from a chick embryo of 12 days which was a part of the E series serial passage. This tooth we will call the test tooth. The other tooth, when removed from Zephiran Chloride, was dropped into bacteria-free allantoic fluid, from a chick embryo of 12 days which had not been injected with dentinal decay filtrate. This tooth was designated as the control tooth. After 2 weeks the tubes containing these teeth were opened with sterile precautions. Additional similar fluid was added. After a total of 32 days the tubes were opened and the teeth removed.

Fig. 4.—Photomicrograph of the tip of a rat's incisor which had been immersed for 32 days in the allantoic fluid of a control chick embryo of 12 days' development. (×80.)

A photomicrograph of the control tooth is shown in Figs. 3 and 4, while a photomicrograph of the test tooth is shown in Figs. 5 and 6.

The pH of the allantoic fluid in which the control tooth was left for 32 days remained at 6.5. The pH of the allantoic fluid containing the test tooth rose to 8.4 during the 32 days when the test tooth was in it.

Around the test tooth on the slide to which it was removed for examination, some crystalline material was deposited. We have just noted the terminal pH of this solution as 8.4. An analysis for calcium in the test allantoic fluid, in which the test tooth had been immersed, showed 5 times the calcium of the control allantoic fluid in which the control tooth had been immersed. It would appear reasonable to credit the greater calcium content of the test solution to calcium salts released from the test tooth.

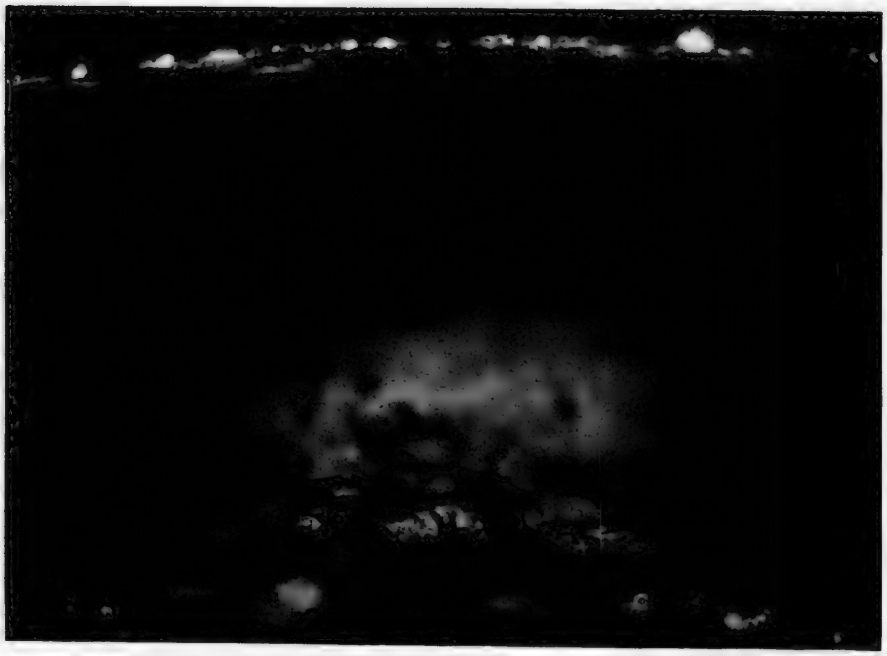

Fig. 5.—Photomicrograph of body of rat's incisor which had been immersed in allantoic fluid of developing chick embryos. The embryos used were part of a serial passage the first of which had been injected with human dentinal decay filtrate. (×80)

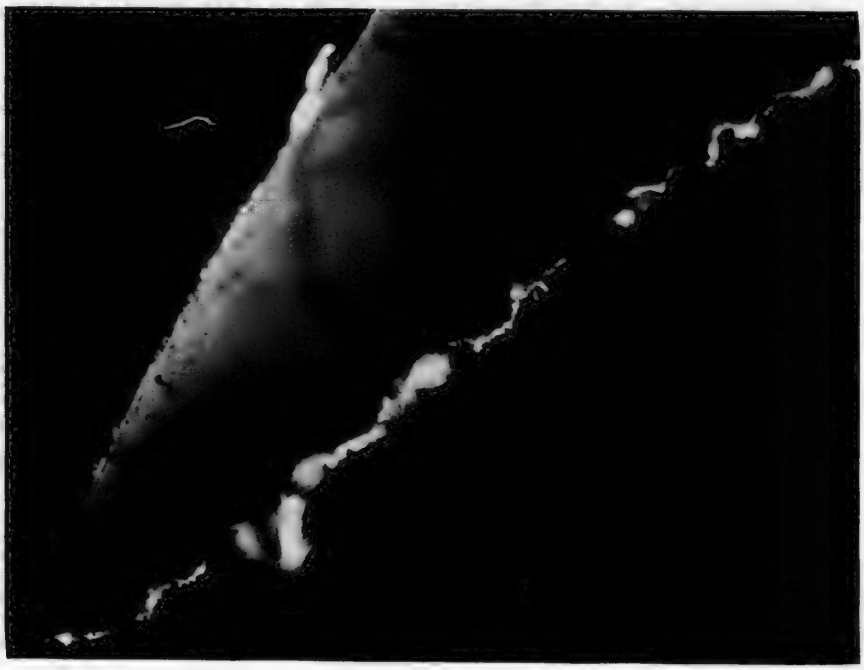

Fig. 6.—Photomicrograph of the tip of a rat's incisor which had been immersed for 32 days in the allantoic fluid of 12-day-old chick embryos who were part of a serial passage starting with human dentinal decay filtrate. Note accretion of crystalline material along the periphery. (×80)

DISCUSSION

It is possible that viral attack on the organic matrix of the tooth may lead toward release of calcium salts from tooth structure. That there is an extensive organic matrix in both enamel and dentin can no longer be seriously doubted.[10, 11] How well this matrix may be expected to support viral growth remains largely for the future to determine. There is some evidence that the organic matrix contains nucleoprotein.[12] Evans[13] pointed to nucleoproteins in bacteriophage reproduction.

Whether the agent considered in this paper is peculiarly adapted to the dentinal environment or whether various viral agents may be associated with dentinal necrosis remains for further work to determine. Some evidence exists that following certain viral diseases there is a change in the enzymic action of the individual's saliva accompanied by increase in dental necrosis.[14]

SUMMARY

Material spooned out from below the floor of a carious lesion and filtered through a Seitz filter was placed upon the chorio-allantoic membrane of chick embryos. The passage of bacteria-free allantoic fluid through a series of chick embryos resulted in the development of inclusion bodies in the chorio-allantoic cells as studied on impression slides.

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ACTINOMYCES AND THE DENTOBACTERIAL PLAQUE

J. ENNEVER, D.D.S., M.Sc.,* H. B. G. ROBINSON, D.D.S., M.S., AND P. C. KITCHIN, M.Sc., D.D.S

Ohio State University, College of Dentistry, Columbus, Ohio

INTRODUCTION

THE role of the dentobacterial plaque in the initiation of dental caries has been well established, if not well understood, for over one-half a century. There are many voids in our information pertaining to this minute, mucilaginous mass of microorganisms and salivary debris. These may be the result of a tendency to magnify effect rather than cause. Evidence for such an interpretation is afforded by the numerous methods presented, especially during the past few years, for the control of dental caries.

This report presents in part the results of a cultural study of the plaque as a biologic entity. Initially, the attention of the investigation centered upon the plaque as a structure using stained sections prepared without disturbing in situ relationships. Information so obtained directed the effort toward determining the occurrence of Actinomyces and the constancy of these microorganisms in the plaque.

LITERATURE

Ficinus,[16] in 1847, noting the consistent occurrence of clumps of microorganisms in association with dental caries, thought there might be a causal relationship. Leber and Rottenstein, whose report of 1873 was cited by Cox,[8] believed that after the enamel surface had been softened by acids, *Leptothrix buccalis* (sic) attached to the enamel and aided further decalcification by concentrating the area of acid activity. In 1886, Black[2] isolated from saliva a coccus which caused a peptonized broth to gel; he offered the suggestion that such activity might facilitate the attachment of other microbial forms to the intact enamel surface. It remained, however, for Williams[40] to demonstrate histologically the existence of, and to establish the significance of, the bacterial plaque in initiating dental caries. He stated ". . . if the environing conditions of the teeth are such as to favor the development and activity of acid producing bacteria, and if these bacteria are permitted to become attached to the surface of the enamel, it is doomed, although it may be the most perfect that was ever formed. On the other hand, if these environing conditions are not present, the worst enamel will not decay." Black[3] was in accord with Williams' views and wrote, "The one thing necessary to the beginning of caries is the formation of such a gelatinous microbic plaque in a secluded position where its acids may act without too frequent disturbance . . . and there give rise to caries." Miller,[26] whose observations had provided the foundation for the chemicoparasitic hypothesis of dental caries, acknowledged

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*Present address: University of Kansas City, School of Dentistry, Kansas City, Mo.

the presence of plaques, but declared, ". . . they are not characteristic of carious teeth. . . ." He later[27] again refused to concede that plaques were an essential element for caries initiation.

For the next several years, there were occasional reports concerning the plaque, the most thorough being that of Lothrop and Gies[24]; but it probably was a result of Dobbs' studies on plaque permeability that interest in the subject was reactivated. Dobbs[10] removed plaques by immersing extracted teeth in 5 per cent hydrochloric acid to which aluminum potassium sulfate had been added to minimize protein alteration. The plaques were then sealed into a diffusion apparatus and various solutes tested for ability to dialyze. He found that the salivary buffers did not pass through the plaque in sufficient quantity to be of significance in controlling caries-producing acids, whereas soluble carbohydrates readily diffused through. Further, on the basis of artificial plaque production, Dobbs concluded that certain oral bacteria alter mucin to a firm gel and this in turn bound salivary debris to form the dentobacterial plaque.

Etherington and Trimble[15] studied plaques from 18 subjects, 6 of whom they considered caries inactive. Determination of pH values of plaque material suspended in distilled water gave a range extending from pH 4.6 to pH 6.8. There was no correlation with caries activity. Stephan[33] also determined pH of water-suspended plaques. The average was pH 5.9 with a range from pH 5.0 to pH 6.8. His later study[34] of plaque pH, in situ, revealed average values of pH 6.8 before a 1 per cent glucose rinse and readings of pH 5.1 after the rinse. These observations, as well as that of Miller, Muntz, and Bradel,[25] amply demonstrated that plaque material was capable of causing carbohydrate degradation with resultant hydrogen ion production. Subsequently, Stephan[35] found that, although there was an increase in acidity of all in vivo plaques after the mouth was rinsed with glucose, the *extent* and *duration* of the pH drop was directly related to the degree of caries activity. A value of pH 5.0 or below occurred only in caries-active mouths. This work has been confirmed and extended by Stralfors.[36]

Direct microscopic examination of plaque materia by Blayney, Kesel, and Wach[4] indicated that plaques associated with carious areas always contained either short parallel rods or coccobacilli, while plaques not associated with caries did not contain these forms. Hemmens, Blayney, and Harrison,[18] continuing the study, proposed that certain nonaciduric microorganisms represented the basic flora of the plaque. The same workers[19, 20] noted a qualitative similarity between the acidogenic flora of saliva and that of the plaques from the same individual. They also reported a marked increase in lactobacilli recovered from plaque material concurrent with the onset of early caries.

Dietz[9] constructed an apparatus with which he was able to observe in vitro plaque formation and caries initiation. He mounted, under high magnification, a section of enamel which was bathed in slowly agitated, fresh human saliva. The plaques Dietz obtained consisted essentially of "yeasts, gram

positive bacilli and filamentous organisms." Bruckner[5] has shown that plaques, qualitatively similar to those in situ formed on strips of Cellophane incubated with fresh saliva at 37° C. Ennever, Robinson, and Kitchin[13] confirmed this observation, found that celloidin gave similar results, and applied the principle to a method for producing plaques in situ suitable for microsectioning without disturbing the natural orientation of the surface and the plaque. The sections at 10 microns disclosed a multitude of long, filamentous, branching structures suggestive of microorganisms. These forms were arranged roughly parallel to each other, approximately at right angles to the celloidin. The branched filaments were often 100 microns or more in length, with irregular outlines, an average diameter slightly greater than 1 micron, and stained uniformly gram-positive. Clumps of other bacteria-like elements were enmeshed by the lengthy forms. Entities not unlike gram-positive rods, cocci, and gram-negative short rods could be distinguished in the interstice. Large, amorphous, stained masses were also observed in the body of the plaque. The interphase between plaque and celloiding pontic was obscured. This was remedied by sections at 8 microns which, in addition to confirming the features of the 10 micron sections, indicated that the filamentous structures were present at the junction of plaque and pontic. The relationship between the pontic and the elongated structures in the plaque suggested that the latter might either afford the attachment of the plaque, its structural framework, or both. Nevertheless, the existence of branched microorganisms in the plaque based solely upon evidence from the sections could only be presumed, for chance arrangement of discrete microbial elements could have simulated branched organisms. Moreover, the structures could have been organic portions of salivary debris and not bacterial cells. However, the consistency of the evidence in scores of sections plus the reported occurrence of Actinomyces in the normal mouth, while not conclusive, gave credence to the microbial interpretation. Based upon this rationale, an investigation of Actinomyces in the dentobacterial plaque was undertaken.

As discussed by Rosebury,[31] the organism most frequently associated with lumpy jaw of cattle was first reported by Harz in 1877, who, from observation of tissue specimens, coined the descriptive term actinomycete and called the disease actinomycosis. The following year, Israel described actinomycosis in man. It was not, however, until 1891 that the organism, an anaerobic parasite, was isolated and described by Wolff and Israel.

Emmons[12] has in addition credited Harz for the specific designation *Actinomyces bovis*. The same author stated ". . . Bostroem also attempted to obtain cultures, and, using aerobic methods . . . isolated in a few of his cultures an aerobic actinomycete . . . which he erroneously described as *A. bovis*." Emmons continued, "*A. bovis* is difficult to isolate and maintain . . . but the Bostroem contaminant grows readily and is easily transferred in culture. As a consequence, few laboratories or culture collections have maintained . . . *A. bovis*, but a strain of Bostroem's aerobic fungus, once deposited in a culture collection under the erroneous name, is maintained easily and re-

mains indefinitely as a source of confusion." To preclude this confusion, Kruse, as cited by Negroni and Bonfiglioli,[29] suggested the specification of *A. israeli* for the parasitic actinomycete associated with actinomycosis. This taxonomic departure was adopted by the Argentine workers as well as by Grooten,[17] Puntoni,[30] and Waksman.[38]

Most English-speaking bacteriologists, nevertheless, have continued to employ the original terminology. Wilson and Miles[41] retained *A. bovis* and suggested *A. gramminis* to indicate Bostroem's organism, while Breed and associates[6] differentiated between *A. bovis* and *A. israeli* on the basis of habitat, colonial structure, and biochemical reactions. Conant and Rosebury[7] designated *A. israeli* as a synonym for *A. bovis*, although, earlier, Rosebury[31] had employed the term *israeli* exclusively. Erickson[14] in a recent review has supplemented *Bergey's Manual* and cited her previous work on the serologic dissimilarity of actinomycetes from human and animal sources.

Although the micro-aerophilic parasitic actinomycetes are placed in one or two species, depending upon the school of thought, it is generally conceded to be a heterogeneous grouping. Erickson wrote, "The extreme sensitivity with which all the actinomycetes react to environmental conditions demands a certain liberality of interpretation in the assignment of specific characters. To turn over the long list of insufficiently described species in Bergey's Manual is to think, not perhaps without a guilty sense of responsibility, that it is surely time that microbiologists . . . build up a body of public opinion which should discourage the (namer of new species) by making him feel the pest that he is."

In view of the existing confusion, it is suggested that the generic term Actinomyces, as proposed by Waksman and Henerici,[39] be used to indicate the anaerobic or micro-aerophilic, non-acid-fast parasitic Actinomyces, and specific designation be omitted until it can be better substantiated. Used in the lower case, actinomyces or actinomycete should be interpreted in the same manner as are the terms mold and yeast.

Soon after the biology of actinomycosis attracted the interest of bacteriologists, Wright[42] predicted that the Actinomyces would be found as members of the normal oral flora. The prediction was verified by the subsequent studies of Lord,[22] Naeslund,[28] Emmons,[11] Lord and Trevett,[23] and Sullivan and Goldsworthy.[37] It is to be noted, however, that no report with the exception of Lord's early morphologic study, proved consistent occurrence of the organisms. Nevertheless, in considering the microbial complexity of source material (saliva, gingival scrapings, and debris from carious teeth) even sporadic recovery should be considered significant. Sullivan and Goldsworthy[37] also noted that Actinomyces, from the normal mouth differed in no important feature from those occurring in actinomycosis. Bibby and Knighton[1] isolated actinomycetes from normal mouths which differed from Actinomyces only in being more oxygen tolerant. Hurst, Frisbie, Nuckolls, and Marshall[21] have studied Actinomyces as cariogenic agents.

Possibly one of the major contributions in recent years to the study of the Actinomyces was that of Rosebury, Epps, and Clark,[32] who developed a method for the isolation of the microorganisms from contaminated sources. A modification of their procedure and the results obtained in a study of the Actinomyces of the dentobacterial plaque are described in the experimental portions of this report.

EXPERIMENTAL MATERIALS AND METHODS

It was deemed advisable to use a single source until isolation procedures were perfected. For the purpose of obtaining plaque material for cultivation, a human lower right first molar tooth was affixed to a removable appliance prepared for a subject whose dentition lacked this tooth. Previous to insertion into the subject's mouth, the proximal surfaces of the experimental tooth were scrubbed for several minutes with a fine, stiff brush until stained smears from the areas did not reveal the presence of filamentous organisms. After remaining in the mouth for periods up to 24 hours, the appliance was removed and the proximal surfaces of the tooth washed in running, sterile water for 90 seconds. With aseptic precautions, plaque was scraped from an area adjacent to the contact point of the tooth and transferred to a micro-mortar containing Triton A-20 at 1:2,000 in 0.05 ml. of sterile physiologic saliva. A coarse suspension of the plaque material was prepared by trituration with a small, sterile glass pestle. One visible granule of the suspension, accompanied by a minimum of the diluent, was transferred by capillary pipette to a brain-heart infusion agar plate. The plate was streaked in one operation, and, without flaming the inoculation loop, 8 additional plates were streaked from the first. The plates were incubated anaerobically in an atmosphere of 5 per cent carbon dioxide for 6 days at 37° C.

Twelve individuals with sufficient posterior diastemata to permit interdental access provided material from which was determined the frequency of occurrence of Actinomyces in a series of plaques. Two of the subjects had accessible surfaces with early caries. Before material was scraped from the interproximal surfaces, each subject rinsed his mouth vigorously with warm, tap water. The procedure employed after removal of the plaque was the same as that described above.

The Actinomyces isolated were studied morphologically and biochemically. For the biochemical characterizations, the basal media for anaerobes, as detailed in the Society of American Bacteriologists' Manual of Methods for Pure Culture Study of Bacteria, leaflet II44, were employed.

RESULTS

By rigid adherence to the isolation procedures described above, 15 attempts to isolate Actinomyces from plaques removed from the contact areas of the human tooth on a removable appliance from one individual were uniformly successful. Conversely, three attempts from the same source, in which a full drop of diluent accompanied the single visible granule during inoculation, failed

to give isolated colonies of Actinomyces. Each cultivation of plaques from the twelve natural dental sources from different individuals was also successful in yielding Actinomyces irrespective of the caries state.

In primary isolation on brain-heart infusion agar, after 6 days at 37° C., anaerobically in the presence of 5 per cent carbon dioxide, the Actinomyces were observed in rough and intermediate colonies. Rough colonies, illustrated in Fig. 1, usually had a diameter somewhat less than 1 mm. In reflected white light, examined with a magnification of ×15, the colonies were glistening,

Fig. 1. Fig. 2.

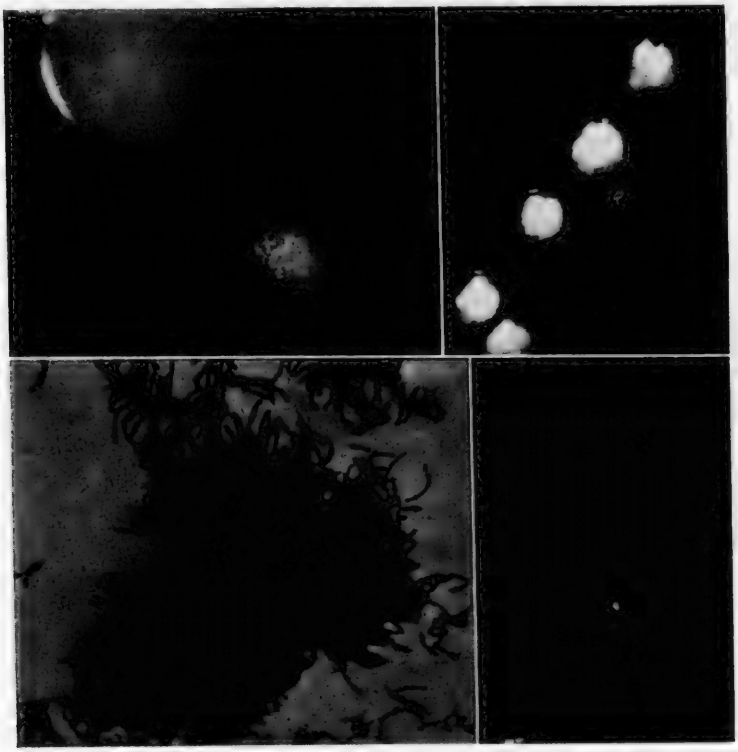

Fig. 3. Fig. 4.

Fig. 1.—Rough colony at ×15 magnification.
Fig. 2.—Intermediate colonies at ×15 magnification.
Fig. 3.—Fixed stained smear (×950 magnification).
Fig. 4.—Dark-field preparation showing branching (×950 magnification).

gray-white and semitranslucent with irregular surfaces and abruptly rising, nonuniform contours. The colonies were hard, adherent, and emulsified with difficulty. Intermediate colonies (Fig. 2) were approximately the same size but with less irregular surfaces and contours. They were softer than the rough colonies, more opaque, and emulsified more readily. As shown in Fig. 3, the most characteristic feature of fixed, stained smears from each colony type

was tangled masses of filaments, many of which appeared branched. The cells were gram-positive, either uniformly so, or with granularity, possessed irregular outlines, and had an average diameter slightly less than 1 micron. Evidence of branching was most convincingly apparent in darkfield preparations (Fig. 4).

The results with most of the biochemical tests attempted were variable among 13 strains studied. Acid without gas was usually produced from glucose, maltose, mannitol, lactose, sucrose, and salicin, after 48 hours, anaerobically. Nitrate reduction varied from strongly positive to negative; and indole, methyl red, acetylmethyl carbinol, hydrogen, sulfide, and gelatin liquefaction were consistently negative.

All strains grew well as large granules in Brewer's thioglycollate within 48 hours. From pH 7.3 to pH 7.5 encompassed the optimum range, and best growth occurred at 37° C. When glucose agar shake tubes were incubated aerobically, growth occurred throughout the tube except for a 1.0 to 1.5 cm. band at the top.

DISCUSSION

The method developed from the isolation of Actinomyces from the dentobacterial plaque differed from that of Rosebury, Epps, and Clark in the physical manipulation of the inoculum. That this simple alteration was significant, however, was evident from the results obtained. Even though the method of Rosebury and his colleagues is adequate for isolating Actinomyces from actinomyoetic pus and other contaminated sources, a series of attempts using this procedure yielded only sporadic recovery of the organisms from plaques. Thus, it was indicated that either the organisms were not consistent components of plaques or else the method was at fault. The constancy of morphologic evidence, from sections and smears, indicated the latter.

Perhaps the success of the modified method was based on the following consideration. Actinomyces tend to persist in tangled masses which resist attempts at emulsification. It was reasoned, therefore, that by trituration of plaque material in a low surface-tension liquid the more readily emulsified cells of the plaque would disperse in the diluent and the Actinomyces would remain clumped. Thus, inoculation of a single visible particle, accompanied by a minimum of diluent, should minimize the opportunity for extraneous microorganisms to obscure and overgrow the Actinomyces. As evidenced, this rationale proved true.

The fact that the organisms were found to be components of plaques, irrespective of the caries relationship, tended to indicate that the mere presence of Actinomyces is not of significance in the cariogenic potential of a given plaque. To further credit this interpretation, it was noted that none of the caries-free surfaces from which Actinomyces were repeatedly isolated became carious during a period of 2 years. Whether or not these organisms are of importance for the plaque attachment or for its structural integrity, remain unanswered questions, the solutions of which are complicated by the constant occurrence of the organism in this portion of the oral flora. However,

morphologic studies strongly suggest that the Actinomyces form the structural frame for the plaque. Although Dietz occasionally observed in vitro plaque formation without detectable filamentous forms, the artificial situation and the limitations of morphologic detection render final judgment impossible. The conclusion of Dobbs is hardly tenable since at acid pH mucin loses its mucilaginous and adherent qualities. The possibility, however, that altered mucin could initiate plaque attachment remains open.

The wide variation in morphologic and biochemical characteristics observed with the 13 strains of Actinomyces studied was in accord with the reports of others. Due to the marked response of these microorganisms to environmental influences, the paucity of reliable information concerning this group, the taxonomic confusion existing at present, and the limited scope of this study, specific designations were not assigned to the Actinomyces of the plaque.

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NITROGEN AND PHOSPHORUS OF EARLY, SMOOTH-SURFACE CARIES OF ENAMEL

THOMAS B. COOLIDGE

Walter G. Zoller Memorial Dental Clinic and Department of Biochemistry, University of Chicago, Chicago, Ill.

THIS paper presents nitrogen and phosphorus analyses of sound human enamel and of the enamel of early, smooth-surface caries before changes of surface contour have occurred.

Analyses of enamel have been reviewed by Leicester[1] and nitrogen analyses of enamel by Losee and Hess.[2] The novel feature of the present analyses is believed to lie in the small amount (1 mg.) of material required for analysis by the methods used, so that comparison is possible between the composition of carious enamel and of noncarious enamel from the same tooth. The analyses were made with the purpose of gaining information on the early stages of the processes producing smooth-surface caries of the enamel.

EXPERIMENTAL

Freshly extracted human teeth were placed in water covered with toluene and stored 1 to 6 weeks. The teeth were rinsed with water, scrubbed with soap and a hard bristle brush, scraped free of debris, scrubbed again, and stored from a few days to a month in distilled water covered with toluene. Before analysis the teeth were dried with absorbent tissue paper. Sound enamel was ground in successive portions from areas of about 8 square millimeters. Carious portions were selected which were colorless, completely homogeneous in appearance, and without change in contour from that of the surrounding enamel. Some were gingival but most were interproximal. Dr. J. R. Blayney confirmed the clinical diagnosis of several of these areas. Carious material, about a milligram in weight, was removed with a delicate hand instrument. Hard white spots (hypoplasia?) of enamel of similar size were removed with a diamond wheel.

All samples were weighed to a precision of about 0.01 mg. immediately after removal. They were then ashed with 2.5 ml. of 10N H_2SO_4 and with H_2O_2 by the method of Koch and McMeekin.[3] Aliquots were analyzed for nitrogen by the method of Conway[4] and for phosphorus by the method of Gomori.[5] Nitrogen analyses were accepted when duplicates within 2 per cent were obtained; phosphorus analyses when duplicates were within 3 per cent. The probable error of the analyses was obtained from 30 analyses of separately weighed samples from 5 pooled lots of normal enamel.

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TABLE I

TOOTH	AREA*	NUMBER OF SAMPLES	PHOSPHORUS GAMMAS/MG.			NITROGEN GAMMAS/MG.			RATIO P/N†		
			MAX.	MIN.	MEAN	MAX.	MIN.	MEAN	MAX.	MIN.	MEAN
Normal	Layer 1	15	224	125	158	12.1	0.40	5.19	367.0	11.9	59.9
Normal	Layer 2	15	259	94	159	11.7	0.27	4.12	388.0	14.7	71.9
Normal	Layer 3	15	225	106	158	11.0	0.88	3.27	179.0	12.4	66.9
Normal	Layer 4	8	214	131	161	11.4	2.13	4.07	76.6	11.5	52.1
Normal	Layer 5	2	146	138	142	2.2	1.28	1.74	108.0	66.4	87.2
Carious	Layer 1	11	237	98	169	14.0	2.82	6.31	51.0	11.1	32.3
Carious	Layer 2	11	196	89	158	7.74	2.11	5.79	76.3	15.1	31.9
Carious	Layer 3	11	293	122	171	11.1	1.23	6.49	154.0	13.0	38.4
Carious	Layer 4	11	298	121	168	28.7	2.89	8.08	45.4	4.93	28.7
Carious	Carious material	11	206	63	143	11.5	2.78	6.16	44.2	11.2	26.3
"Hypoplastic"	Layer 1	4	203	64	132	11.3	3.37	6.08	36.0	12.2	24.5
"Hypoplastic"	Layer 2	4	189	124	155	8.8	3.16	5.39	46.6	14.1	34.1
"Hypoplastic"	Layer 3	4	200	102	164	6.39	2.45	3.86	69.4	16.0	50.4
"Hypoplastic"	Layer 4	4	186	114	152	11.8	3.38	5.87	51.5	9.63	34.9
"Hypoplastic"	Hypoplastic material	4	153	54	117	8.12	2.86	5.02	53.5	14.3	27.2

*All areas marked "layer" are from sound enamel.
†P = phosphorus; N = nitrogen.

RESULTS

The probable error of the nitrogen analyses, including weighing, was 0.95 per cent; that of the phosphorus analyses, 1.4 per cent. The results of the analyses are given in Table I. *Layer 1* represents the layer of enamel removed first; *Layer 2* that removed second, etc., all "layers" being removed from apparently normal areas.

DISCUSSION

The analyses show that there is a large variation in the nitrogen and phosphorus content of enamel from different teeth and from different areas of the same tooth. They show no consistent differences between normal enamel, carious enamel, and hard white spots of enamel. The outermost layers of normal enamel show a higher nitrogen content in general than deeper layers as might have been anticipated from the appearance of a surface membrane on decalcification of teeth with mineral acids.

It is to be noted that a loss of phosphorus from enamel in the carious process would not be entirely reflected in phosphorus analyses of the removed material were there either (1) a decrease in density of the enamel associated with caries, or (2) a more rapid loss of water, before weighing from the removed carious enamel than from normal enamel. Insofar as a decrease in density was due to replacement of apatite by water, such a decrease would not entirely eliminate a drop in phosphate as measured in removed material. Loss of weight of the carious material during weighing has not been observed. The phosphorus analyses do not show a large general removal of phosphate in early smooth surface caries of the enamel.

With similar considerations as to density change and water loss obtaining, the figures for nitrogen indicate that any nitrogenous material lost by proteolysis and diffusion is balanced by replacement of nitrogen from some such source as saliva.

The phosphorus to nitrogen ratios are not subject to the above considerations as to density and water loss. They give no evidence for a preponderance of either phosphorus or nitrogen removal in the type of caries examined.

SUMMARY

Phosphorus and nitrogen analyses of normal enamel and of the enamel of early smooth surface caries show no large general differences in phosphorus or nitrogen content.

All the analyses were performed by Miss A. E. I. Ebisch. The teeth were supplied through the courtesy of Dr. Eli Olech of the University of Illinois College of Dentistry.

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THE PRODUCTION OF ACIDS FROM GLUCOSE BY ORAL MICROORGANISMS: LACTIC AND PYRUVIC ACIDS

ISAAC NEUWIRTH AND WILLIAM H. SUMMERSON*

Department of Pharmacology and Therapeutics, New York University College of Dentistry, New York, N. Y., and Department of Biochemistry, Cornell University Medical College, New York, N. Y.

IT WAS first shown by Neuwirth and Klosterman[1] that there is a rapid production of lactic acid in the oral cavity when certain carbohydrates are allowed to dissolve in the mouth, and that this was probably due to the action of oral microorganisms, since it likewise occurred when saliva was incubated in vitro with carbohydrate, but not if the saliva had first been rendered sterile by passage through a Seitz filter. It was also shown in a few experiments that when saliva was incubated with glucose solutions for one-half hour, only about one-third of the glucose disappearing could be accounted for as lactic acid.

These results raised a number of important questions. Did the rate and extent of glucose metabolism in saliva vary from one person to another? Was lactic acid the sole acid formed, or was it one of several acids which made up the total acid production? Was lactic acid an end-product or an intermediate stage in the degradation of glucose by oral microorganisms under aerobic and physiologic conditions? What bearing, if any, did the answers to these questions have on the problem of dental caries?

The experiments reported here represent attempts to answer the various questions raised. Briefly, we have investigated the action of saliva, obtained from a variety of subjects, on added glucose under physiologic conditions of temperature, CO_2 tension, and pH, in quantitative terms of glucose utilization, total acid production, production and disappearance of specific acids (lactic, pyruvic), as well as certain interrelationships between the metabolism of glucose, lactate, and pyruvate by mixed oral microorganisms.

METHODS

Saliva was collected in a small beaker, by paraffin stimulation, 2 to 3 hours after the last meal, usually breakfast. As a rule, from 5 to 10 minutes were required to collect about 10 ml. of saliva. The subject was instructed to chew the small piece ($10 \times 10 \times 15$ mm.) of paraffin vigorously and to shift it about in the mouth in order to dislodge organisms from the teeth.

Incubation with added glucose, and manometric measurement of oxygen consumption, respiratory CO_2 production, and total acid production were carried out using the Summerson differential manometer and vessels.[2] Duplicate 2.0 ml. portions of the unfiltered saliva were measured into the two vessels belonging to a single manometer, and 0.3 ml. of 1 per cent glucose solution was placed in a side bulb of each vessel; 0.2 ml. of 2.5N hydrochloric acid was placed in a second

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*Present address: Biochemistry Section, Medical Division, Army Chemical Center, Md.

side bulb of each vessel, attached to the vessel at right angles to the first side bulb. The vessels were fastened to the manometer, placed in a water bath at 38° C., and filled with a gas mixture containing 5 per cent CO_2 and 95 per cent O_2 by passing the gas through the vessels equilibrated at atmospheric pressure for 10 minutes. The vessels were then closed off to the air, so that pressure changes within the vessels could be measured on the manometer.

One of the two vessels attached to the manometer served as control, the other was the experimental vessel. To start an experiment, the acid was tipped into the saliva from a side bulb for the control vessel stopping all bacterial action instantaneously, and the glucose solution was simultaneously tipped into the saliva in the experimental vessel. After incubation with shaking for the desired length of time (usually 15 minutes for one manometer and 30 minutes for a second manometer, both charged with saliva from the same subject) bacterial action in the experimental vessel was stopped by running in the acid from the second side bulb. At the same time, the glucose solution in the control vessel side bulb was mixed with the acidified control vessel contents, thus permitting establishment of the exact amount of glucose present at the start of the experiment by analysis of this mixture.

Manometric readings were made during the incubation period, and subsequent to it, in the same fashion as has been described for similar measurement on isolated tissue slices.[2] From these readings were obtained the values for oxygen consumption, respiratory CO_2 production, and total acid production* during the incubation period. The pH of the saliva before and after incubation with glucose was also obtained from manometric readings. When acid is tipped into the control sample of saliva at the beginning of the experiment, the pressure increase which is readable directly on the manometer is a measure of the bicarbonate content of the sample. From this value, and the known (5 per cent) CO_2 content of the gas phase, the pH is readily calculated by appropriate use of the well-known Henderson-Hasselbalch equation (see Dixon[3] for details). After the incubation period, the manometric readings themselves are measures of any change from the initial values in bicarbonate content and CO_2 tension. From knowledge of such changes, the final pH is likewise readily calculated. After the manometric measurements were completed, the contents of each vessel were quantitatively transferred to a glass-stoppered tube graduated at 10 ml., the volume at this point being about 6 ml. Deproteinization was carried out by the Somogyi procedure[4]: 0.5 ml. of 10 per cent $ZnSO_4$ solution was added to the contents of the tube, followed by a drop of 0.025 per cent phenolphthalein solution; 0.5N NaOH solution was then added to faint alkalinity, the contents of the tube made up to 10 ml. with H_2O, well shaken, transferred to a 15 ml. centrifuge tube, and centrifuged for 5 minutes. Suitable aliquots of the supernatant fluid were analyzed for glucose by the Benedict method[5] and for lactic acid by the Barker-Summerson method[6] and for pyruvic acid (when necessary) by the method of Penrose and Quastel.[7]

*Since saliva contains other buffers in addition to bicarbonate, and acid produced reacts in part with these buffers, the extent to which this occurs must be established for each sample, and a suitable correction applied to the manometric readings. This was done by the method of Dickens.[17]

Except where otherwise specified, all analytical results represent the difference between control and experimental samples of saliva, that is, the increment or decrement produced experimentally.

RESULTS

The results of the first series of experiments are summarized in Table I. For the purposes of comparison, the subjects have been divided into two groups. Group I comprises those subjects in whom careful dental examination at the time of the experiment revealed no evidence for the presence of active caries.* Certain of these subjects had apparently never had caries at all; others showed evidence of caries in the past, but the disease was considered inactive at the time of the examination. This group contained 2 children, the other 10 subjects were adults. The second group of subjects, Group II, consisted entirely of children under 10 years of age, in all of whom the caries process was active at the time of the experiments.

TABLE I

METABOLISM OF GLUCOSE BY ORAL MICROORGANISMS IN SALIVA AS MEDIUM

(1) EXPERIMENT NO.	(2) AGE (YRS.) AND SEX	(3) OXYGEN CONSUMED (C.MM.)	(4) GLUCOSE METABOLIZED (G) γ	(4) GLUCOSE METABOLIZED (G) %	(5) TOTAL ACID (T) (C.MM.)	(6) LACTIC ACID PRODUCED (LA) γ	(6) LACTIC ACID PRODUCED (LA) (C.MM.)	(7) LA/G (%)	(8) LA/T (%)
Group I (Active Caries Not Present)									
4	35 M	57	400	13.2	---	137	34	34.2	---
22	27 M	59	285	9.5	23	48	12	16.8	52.2
21	22 M	74	580	17.8	70	148	37	25.5	52.9
24	45 M	87	441	14.4	---	45	11	10.2	---
5	22 F	92	940	31.1	146	281	70	29.9	47.9
7	30 M	157	1280	40.4	197	403	101	31.5	51.2
6	21 F	160	1090	35.7	144	252	63	23.1	43.7
3	22 M	198	1200	40.6	219	421	105	35.1	47.9
1	45 M	219	1180	40.4	194	301	75	25.5	38.6
25	9 M	246	1710	56.4	243	520	130	30.4	53.4
8	38 M	268	2400	79.6	395	910	228	37.9	57.7
18	8 M	347	2295	75.0	338	392	98	17.1	29.0
Group II (Medium or Marked Active Caries)									
14	9 M	70	360	11.9	52	93	23	25.8	44.8
23	8 F	77	470	15.4	63	88	22	18.7	34.9
13	8 M	119	750	25.0	78	124	31	16.5	39.7
15	7 F	129	860	27.0	128	184	46	21.4	35.9
12	10 M	135	1020	32.9	163	292	73	28.6	44.8
28	7 F	140	960	32.0	114	228	57	23.7	50.0
16	9 M	156	1240	39.5	199	344	86	27.7	43.2
11	10 F	175	940	30.5	140	129	32	13.7	22.8
17	7 F	186	1020	33.2	186	134	34	13.1	18.3
9	8 M	290	1490	49.4	171	159	40	10.7	23.4

Within each group, the subjects have been listed quite arbitrarily in the order of increasing oxygen consumption by salivary microorganisms during the experimental period. No significance is to be attributed to this choice, other than as a rough index of the intensity of aerobic metabolism in the

*We are indebted to Dr. Samual S. Wald for most of the reports on the dental condition of our subjects. Clinical and roentgenographic examinations were made.

specimen of saliva, determined not only by the number of microorganisms present but also by their type. It is interesting to note, however, that the rate of glucose metabolism roughly parallels the rate of oxygen consumption.

Considering the data of Table I as a whole, without reference to the presence or absence of active caries, several facts become evident. During the 30-minute incubation period, oral microorganisms are capable of metabolizing from 10 per cent to 80 per cent (approximately) of the glucose present, the average value for 22 experiments being about 34 per cent of the added glucose, at an initial glucose level of 0.13 per cent. There is a rough parallel between the rates of glucose metabolism and of oxygen consumption, indicating that the organisms are deriving oxidative energy from glucose combustion, at least in part. That this is not the whole story, however, is shown by the results of 5 experiments, not included in Table I, in which oxygen consumption in the absence of added glucose averaged 70 per cent of that found when glucose was present; furthermore, as will be shown later, oxygen consumption in the presence of added lactic acid alone is practically identical with that found in the presence of glucose.

The quantitative relationship between glucose metabolism and lactic acid formation is shown in Column 7 of Table I. From 10.2 per cent to 37.9 per cent of metabolized glucose gives rise to lactic acid; in 22 experiments, an average of 23.5 per cent of the glucose disappearing is found as lactic acid. Further aspects of this relationship will be considered later.

The contribution of lactic acid formation to the total acid produced during glucose metabolism is shown by the data of Column 8, Table I, which is the ratio of the values given in Column 6 (expressed as c.mm.) to those in Column 5, the latter being a measure of the total acid produced during an experiment, expressed as c.mm. of CO_2 liberated from bicarbonate by the neutralization of the formed acid. It is seen from the data of Column 8 that from 18.3 per cent to 57.7 per cent of all the acid formed is accountable for as lactic acid, with an average value of 41.6 per cent for 20 experiments. In other words, over one-half of the acid produced when oral microorganisms are incubated with glucose in saliva may not be lactic acid.

A consideration of the data of Table I with regard to any possible relation to caries fails to reveal any significant influence of this disease on the quantities we were measuring. With respect to active caries, our subjects fall into two clear-cut groups, regardless of any niceties in diagnosis. The subjects in Group II showed evidence of active caries; those in Group I did not. Yet there is no significant difference between the two groups of subjects with regard to bacterial activity against glucose. The values for rates of oxygen consumption, glucose metabolism, and total acid production overlap for the two groups of subjects, in fact, the highest values are found in the noncarious group. The fraction of glucose metabolized which appears as lactic acid (Column 7) averages 26.4 per cent for Group I and 23.5 per cent for Group II; the difference between these values is not significant. The corresponding values for the fraction of the total acid which is identifiable as lactic acid

(Column 8) are, respectively, 47.5 per cent and 35.8 per cent for the subjects in Groups I and II. We do not believe this difference between these latter two values is significant, as will be made clear from later experiments.

At the same time, it is clear that there is one major difference between the subjects in Groups I and II of Table I, and that is in age distribution; all those in Group II are children, while all except 2 in Group I are adults. Because of the possibility that such age differences might enter into our results, we felt it desirable to plan a second series of studies in which age differences were less important a factor. Furthermore, we wished to extend our studies in several ways. In our first series of experiments, no attempt was made to establish the bacteriologic aspects of our saliva samples, but it became clear that such knowledge would be of aid in interpreting results. Provision, therefore, was made for the determination of total bacteria and lactobacilli count on our samples at the time of use.*

We likewise analyzed the samples in this series for their pyruvic acid content as well as for lactic acid. Preliminary experiments on a few of the saliva samples represented in Table I had revealed the presence of detectable amounts of pyruvic acid after 30 minutes' incubation, although little or none was found in shorter periods. It appeared possible that the difference we obtained between total acid and lactic acid might be accounted for as pyruvic acid, at least in part.

The results of this second series of experiments are presented in Table II; like Table I, the subjects are divided into two groups. The subjects in Group I were carefully selected for complete absence of any signs of caries, either past or present, using x-ray examination to corroborate the diagnosis. The subjects in Group II either had caries at the time of the experiments or showed ample evidence of having had caries in the past. The results for both children and adults are presented together. The total number of subjects is small, chiefly because of the difficulty of obtaining satisfactory subjects for inclusion in Group I.

The characteristics of the saliva obtained from the subjects used in this series of experiments are presented in the upper portion of Table II. The values for lactic acid, pyruvic acid, pH, and bicarbonate content are those prevailing in the sample at the beginning of the experimental period, prior to the addition of substrate, with the saliva at a temperature of 38° C. and in equilibrium with an atmosphere containing 5 per cent CO_2.

It will be noted that the bicarbonate content of the saliva varies widely, over the range from 17.9 to 66.0 volumes per cent, with a corresponding variation in pH from 6.96 to 7.53. There is no significant difference between the two groups of subjects in either pH or bicarbonate content. This lack of apparent

*Grateful acknowledgment is made to Dr. John F. Reinhard for the bacteriologic studies. Lactobacilli were determined by the dilution pour method on 0.1 and 0.2 ml. samples of saliva, using Difco tomato juice agar, with added agar to make 2.5 per cent final concentration, and adjusted to pH 5 with lactic acid. Counts were made after 4 days' incubation at 37° C. For the total count, 0.1 ml. portions of serial dilutions of saliva in physiologic saline were employed for pour plates, using Difco veal infusion agar, with agar added to make 1.5 per cent final concentration, and with 0.5 ml. of oxalated rabbit blood added to each tube of medium just before pouring. The total count as given does not include lactobacillus colonies.

Attention is called to the fact that many investigators (see, for example, reference 18) regard streptococci and not lactobacilli as the important acid-producing organisms in the mouth.

correlation between salivary pH under our experimental conditions and the presence or absence of active caries has been a consistent finding in all our experiments.

The lactate and pyruvate content of the various samples of saliva as collected is quite low, and here again there are no differences between the two groups of subjects. The total bacteria count varies widely from subject to subject, as would be expected; the group of subjects with caries (past or present) has in general the higher bacterial count, but one of the noncarious subjects showed a higher count than two of the caries group.

The major bacteriologic difference between the two groups of subjects is in the lactobacillus count. Lactobacilli were not found in the saliva from any of the subjects in Group I; they were present for all subjects in Group II.

The metabolism of glucose and the production of acid by salivary microorganisms for the subjects in this series of experiments is shown by the data in the lower portion of Table II. Considering the data as a whole, without reference to the division of subjects into two groups, it is evident that from 14.0 per cent to almost 47 per cent of the added glucose was metabolized during the 30-minute experimental period. Glucose metabolism parallels in general the rate of oxygen consumption; this latter value, however, bears little or no relationship to the total bacteria count, as would be expected, since there would appear to be no predictable basis for distinguishing between organisms which have primarily an oxidative type of metabolism and those with a fermentative type, in the mixed bacterial flora of ordinary saliva.

Acid production from glucose ranges from 70 to 288 cmm. of CO_2 per 30 minutes; acid production in general parallels oxygen consumption, again with certain exceptions. Of the total acid produced, from about 23 per cent to about 64 per cent can be accounted for as lactic acid. This lactic acid in turn represents from 13.5 per cent to 42.0 per cent of the glucose which is metabolized.

The values for pyruvic acid production are of interest. Significant production of pyruvic acid was found in all the samples analyzed, ranging from 18.0 per cent to 35.6 per cent of the total acid production, and accounting for from 10.3 per cent to 17.3 per cent of the metabolized glucose. It must be stressed here that this is for a 30-minute incubation period; as will be shown later, pyruvic acid production is to a significant extent a function of the incubation time.

When the contribution of both lactic acid and pyruvic acid to the total acid production is evaluated, it is seen that in one case (subject F. M.) all of the acid produced can be accounted for in this way. For the other subjects, lactic acid and pyruvic acid together account for from 43.8 per cent to 78.5 per cent of the total acid produced. This is, therefore, additional evidence that in general some acid or acids other than lactic acid and pyruvic acid are produced by the action of salivary microorganisms on glucose, and in significant amounts. Further evidence on this subject will be presented subsequently.

Now, considering the data of Table II with respect to any differences between the caries-immune and carious groups of subjects, it is clear that there

TABLE II

PRODUCTION OF LACTIC AND PYRUVIC ACIDS FROM GLUCOSE BY ORAL MICROORGANISMS IN SALIVA AS A MEDIUM

	GROUP I (CARIES IMMUNE)				GROUP II (CARIOUS)				
SUBJECT:	F. M.	L.	G. F.	L. V.	W. S.	J. R.	L. M.	R. V.	H. S.
AGE (YEARS):	7	ADULT	8	7	ADULT	ADULT	9	9	7
SEX:	M	M	F	M	M	M	M	M	M
Characteristics of Saliva									
pH	7.25	7.53	6.96	7.18	7.24	7.44	7.43	7.35	7.19
Bicarbonate content; vols. per cent (as CO_2)	35.1	66.0	17.9	29.5	33.6	54.2	52.8	43.4	30.6
Initial lactic acid content, mg. per cent	1.5	1.6	1.0	1.8	2.9	1.9	1.5	1.2	1.3
Initial pyruvic acid content, mg. per cent	0.4	---	0.1	----	0.4	0.6	0.0	0.0	0.4
Lactobacilli per c.c.	0	0	0	0	250.0	25.0	300.0	1040.0	----
Total bacteria per c.c., $\times 10^6$	1310.0	38.0	4.0	50.0	910.0	6200.0	360.0	6000.0	----
Production of Acids From Glucose (3000 μg. glucose added to 2 c.c. saliva; 38° C.; 30 minutes)									
Oxygen consumed, c.mm.	118.0	123.0	189.0	238.0	65.0	97.0	126.0	158.0	234.0
Glucose metabolized:									
Micrograms	790.0	605.0	880.0	1205.0	419.0	640.0	760.0	1020.0	1400.0
Per cent of added glucose	26.3	20.2	29.3	40.2	14.0	21.3	25.3	34.0	46.8
Total acid produced, as c.mm. of CO_2	90.0	150.0	138.0	178.0	70.0	151.0	182.0	208.0	288.0
Lactic acid produced:									
Micrograms	231.0	136.0	151.0	163.0	176.0	238.0	273.0	313.0	295.0
As c.mm. of CO_2	58.0	34.0	38.0	41.0	44.0	60.0	68.0	78.0	74.0
Per cent of total acid	64.4	22.7	27.5	23.0	62.8	39.5	37.3	37.5	25.7
Per cent of glucose metabolism	29.3	22.5	17.2	13.5	42.0	37.2	36.0	30.7	21.1
Pyruvic acid produced:									
Micrograms	127.0	---	152.0	----	43.0	110.0	116.0	150.0	209.0
As c.mm. of CO_2	32.0		38.0		11.0	28.0	29.0	38.0	52.0
Per cent of total acid	35.6	---	27.5	----	15.7	18.6	15.9	18.3	18.0
Per cent of glucose metabolism	16.1	---	17.2	----	10.3	17.2	15.3	14.7	14.9
Per cent of total acid as lactic + pyruvic	100.0		55.0		78.5	58.2	53.4	55.8	43.8

is no sharp distinction between the two groups of subjects with respect to the action of salivary microorganisms on glucose. The range of values for each of the quantities measured is approximately the same for each group of subjects. We must conclude, therefore, that our data afford no evidence thus far for any influence of the caries process on the action of oral microorganisms in saliva on glucose, under physiologic conditions of temperature and pH.

We next investigated the question of why lactic acid contributed only about 50 per cent or less of the total acid production in the majority of our experiments. One explanation for this appears to be the possibility that lactic acid once formed from glucose is further acted upon, either by the same organism or more probably by other organisms present, to produce some substance which might or might not be acidic. It has already been mentioned that in earlier experiments we had noted in a few instances that pyruvic acid production did not begin concomitant with lactic acid production, which begins immediately on the addition of glucose to the saliva, but rather appeared to depend upon the prior production of significant amounts of lactic acid. This would indicate that lactic acid is further metabolized in saliva, giving rise in part to some pyruvic acid. Accordingly, the action of salivary microorganisms on lactate as substrate was studied.

For these experiments, the same group of subjects used to obtain the data of Table II were employed, and the experimental conditions were identical with those already described, except for the use of sodium dl-lactate as substrate instead of glucose. The lactate was added at a level equivalent to 500 μg. of lactic acid per 2 ml. of saliva, and the incubation time was 30 minutes. At the end of this time, the saliva was analyzed for lactate and pyruvate; measurements of oxygen consumption were also made. The results are given in Table III.

TABLE III

THE METABOLISM OF LACTATE BY ORAL MICROORGANISMS IN SALIVA AS MEDIUM*

SUBJECT	OXYGEN CONSUMED (C.MM.)	LACTATE METABOLIZED (LA) γ	LACTATE METABOLIZED (LA) %	PYRUVATE FORMED (PA) (γ)	PA/LA (%)
F. M.	120	93	20.9	33	35.5
L.	119	142	33.4	--	---
G. F.	187	234	54.0	72	30.7
L. V.	222	233	54.0	--	---
W. S.	63	64	13.6	40	62.5
J. R.	105	93	20.9	41	44.2
L. M.	100	70	15.6	47	67.3
R. V.	182	216	50.4	55	25.5
H. S.	207	299	70.3	31	10.3

*500 μg. lactic acid as sodium dl-lactate added to 2.0 c.c. of saliva after preliminary equilibration at 38° C. in presence of 5 per cent CO_2-95 per cent O_2 gas mixture. Experimental period: 30 minutes.

These results show quite clearly that lactate in saliva is rapidly metabolized, and that pyruvic acid is a significant result of such metabolism of lactate. From 13.6 per cent to 70.3 per cent of the added lactate disappeared during the 30-minute incubation period, and from 10.3 per cent to 67.3 per cent of the

metabolized lactate appeared as pyruvate. Oxygen consumption by salivary microorganisms in the presence of lactate as substrate covers a range similar to that found for glucose. There are no apparent differences between the caries saliva and caries-immune saliva with respect to either oxygen consumption, rate of lactate metabolism, or rate of pyruvate formation from lactate.

Our next experiments dealt with the time relationship between the rates of formation and disappearance of lactic acid, pyruvic acid, and total acid, when saliva containing oral microorganisms was incubated with added glucose. Three such experiments were carried out. For each experiment, a series of samples of the subject's saliva was brought to physiologic conditions of temperature and pH by our usual procedure, and then 500 μg. of glucose added to each sample. At suitable time intervals thereafter, a sample was removed from the thermostat, treated with acid as usual to stop metabolic processes, and analyzed for lactic and pyruvic acids. In two experiments, certain of the samples were incubated in the vessels of the differential manometer so that total acid production values would be obtained. The results of these experiments are summarized in Table IV. .All results in this table are expressed in terms of c.mm. of CO_2 equivalent to the amounts of lactic and pyruvic acids present, so that direct comparison of these values may be made with the values for total acid production, which are in the same units.

TABLE IV

RATE OF FORMATION AND DISAPPEARANCE OF LACTIC ACID, PYRUVIC ACID, AND TOTAL ACID, WHEN SALIVA CONTAINING ORAL MICROORGANISMS IS INCUBATED WITH ADDED GLUCOSE*

	TIME IN HOURS AFTER ADDITION OF GLUCOSE						
	0.0	0.5	1.0	1.5	2.0	2.5	3.0
Experiment 1							
Subject C							
Lactic acid	9	45	26	10	9		
Pyruvic acid	6	24	36	40	42		
Experiment 2							
Subject F							
Lactic acid	39	78	45	21	14		
Pyruvic acid	15	38	72	90	80		
Total acid	0	152			118		
Experiment 3							
Subject W							
Lactic acid	8	19	14		7		6
Pyruvic acid	2	19	17		3		2
Total acid	0	88					65

*Glucose added: 500 μg. to 2.0 c.c. saliva. The glucose was completely metabolized during the first one-half-hour period in each experiment. All acid values are in terms of c.mm. CO_2 equivalent to acid present.

It can be seen from the data of Table IV that lactic acid production and pyruvic acid production are significant within the first one-half hour after the addition of glucose to the saliva, the rate of lactic acid production being slightly more rapid. The peak of lactic acid production is reached at the one-half-hour period; at this time all of the added glucose has been metabolized. In the absence of glucose, the lactic acid present in the saliva rapidly disappears, and after 2 hours it is back to the control level or below in all three experiments.

The course of pyruvic acid production is quite different. Pyruvic acid appears to be formed only a little less rapidly than is lactic acid for the first 30 minutes, in two experiments, and even more rapidly in the third experiment. However, after lactic acid formation has ceased, pyruvic acid production continues throughout the remainder of the incubation period in two of the three experiments. In these two experiments the sustained increase in pyruvic acid content as the lactic acid content decreases leads to the conclusion that the pyruvic acid is being formed at the expense of the lactic acid. It will be recalled that the data of Table III demonstrate that pyruvate can be formed from lactate by oral microorganisms. In the case of the third experiment, it is concluded that organisms capable of metabolizing both pyruvate and lactate at approximately the same rate must be present. Experiments in which sodium pyruvate was used as substrate demonstrated conclusively that pyruvate is readily metabolized by oral microorganisms, with no concomitant production of lactic acid.

The data likewise show that the total acid production is a function of the time of incubation. In Experiment 2 the total acid production value decreased from 152 to 118 c.mm. in 2 hours, and in Experiment 3 from 88 to 65 c.mm. in 3 hours. It is interesting to note that at the 2-hour period in Experiment 2 pyruvic acid accounts for most of the total acid still present, while in Experiment 3 neither lactic nor pyruvic acids represent any significant proportion of the total acid present after 3 hours' incubation.

DISCUSSION

Most of the data presented have been for an incubation period of 30 minutes. In our manometric work, many of our experiments were run for both 15-minute and 30-minute periods. The results for 15-minute incubation periods have been omitted from the tables for reasons of brevity and clarity, but they show quite clearly that the action of salivary microorganisms on glucose is substantially a linear function of the time of incubation with respect to oxygen consumption, glucose utilization, lactic acid production, and total acid production, provided there is an excess of glucose present. Once the glucose present has been metabolized, the data of Table IV show that, in general, the course of events tends to be reversed. There is a decrease in the amount of lactic acid present, as well as a decreased total acid level. Only with respect to pyruvic acid formation is there evidence that this is not directly dependent upon the presence of glucose in the medium.

We have also observed in a number of experiments that lactic acid production is significant within a few minutes after contact between the microorganisms and the glucose-containing solutions. We conclude, therefore, that the conversion of glucose into lactic acid and other acids by oral microorganisms is an immediate and direct process and that it continues substantially at the initial rate until all or nearly all of the glucose present has been metabolized. In most of our experiments, the initial glucose concentration was approximately 0.13 per cent. This is slightly above the average value for the

blood and other body fluids, but is considerably lower than that which is found in carbohydrate-rich foods. If such food material were to be held within the oral cavity for more than a minute or two, in contact with saliva, our data indicate that a significant proportion of the glucose present might appear as lactic acid. Perhaps it is such a rapid production of lactic acid which accounts for the fall in pH reported by Stephan.[8]

When we inquire into the possible significance of such lactic acid formation on dental caries in the mouth, however, the importance of lactic acid per se appears to be minimized rather than enhanced. We have presented ample evidence that other acids than lactic acid are formed during the action of salivary microorganisms on glucose under physiologic conditions, since under ordinary circumstances lactic acid accounts for only a part of the total acid formation. Some of this other acid fraction has been shown to be pyruvic acid, but we feel that pyruvic acid formation is secondary to the formation of lactic acid, and that pyruvic acid, in general, is not an important end product of brief contact between oral microorganisms and glucose. This view is contrary to that of Fosdick and Stark[9] who reported lactic acid formation from pyruvic acid by *L. acidophilus*. Fosdick and Wessinger[10] also regarded pyruvic acid as important in the etiology of dental caries. However, the experimental conditions used by Fosdick were distinctly different from and, in our opinion, less physiologic than our own. (See also in this connection references 11-13.)

The nature of the extra acid or acids produced has thus far not been elucidated. Evidence has been presented elsewhere[14] that citric acid is not a component of the total acid production. We have attempted to establish the presence of acetic acid, but without success. Analytical methods available for the determination of small amounts of acetic acid in biologic material are, in general, unsatisfactory, and it is possible that improved methods might permit the inclusion of acetic acid in our total acid fraction. In this connection it is of interest to note that Muntz[15] reported finding, besides lactic acid, acetic, propionic, and formic acids from the incubation of glucose with dental plaque material. It is well known, of course, that a variety of organic acids, such as lactic acid, pyruvic acid, acetic acid, propionic acid, butyric acid, etc., are end products of the action of various microorganisms on glucose.

It would appear that a systematic study of the various possibilities under our experimental conditions might permit the attainment of a reasonably complete picture for the distribution of total acid between various known components. One might, however, question the practical value of such a study from the point of view of tooth decay. Our data consistently fail to reveal any influence of even markedly active caries on the quantities we were measuring. While there are wide variations between individuals with respect to the quantitative aspects of the action of oral microorganisms on glucose, the same range of values is found for those individuals with even markedly active caries as for those individuals who either show no signs of ever having had the disease or in whom the disease is latent. We can only conclude that if there is any relation between caries and carbohydrate metabolism by oral microorganisms, such

relation is not evident by the experimental approach we employed. An identical conclusion was reached by Miller, Muntz, and Bradel[16] in their study of the decomposition of carbohydrates by dental plaque material.

SUMMARY

1. The action of oral microorganisms on glucose, in saliva as a medium and under physiologic conditions of temperature, CO_2 tension, and pH, has been studied for a variety of human beings, with respect to the rate and extent of action on glucose, the amount and type of acid produced, and the quantitative and temporal relation between glucose metabolism and acid production under these conditions.

2. Immediately upon contact between glucose and saliva, the microorganisms present initiate a rapid and sustained attack upon the glucose, so that in some instances as much as 80 per cent of the glucose added at a level of 0.13 per cent will disappear in 30 minutes.

3. One major result of the action of oral microorganisms on glucose is the production of lactic acid. However, for most subjects studied the lactic acid formed accounted for less than one-half of the total acid production as measured manometrically. Some pyruvic acid was occasionally formed, but in the majority of subjects lactic and pyruvic acid production did not account for more than one-half to two-thirds of the total acid production. The nature of the remaining acid or acids is not known.

4. In the mixed flora of the saliva, lactic and pyruvic acids are metabolized as well as produced. Hence, the net amount of acid present at any given time due to bacterial action on glucose represents a balance between rate of formation and rate of destruction.

5. Consideration of our results with respect to the presence or absence of active caries in the subjects studied failed to reveal any influence of this disease on the quantities measured.

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PENICILLIN RESISTANCE OF STREPTOCOCCI AND STAPHYLOCOCCI IN A PENICILLIN DENTIFRICE STUDY

H. E. LIND, PH.D., AND H. A. ZANDER, M.S., D.D.S.

Brooks Hospital, Brookline, Mass., and Tufts College Dental School, Boston, Mass.

A CLINICAL study was conducted on the effect of a penicillin dentifrice on caries incidence in school children.[1] The children, in grades 1 to 6 of two elementary schools in Walpole, Mass., brushed their teeth once daily, under supervision of the teacher or the hygienist, with a tooth powder which they were given. The children in the control school were given a tooth powder which contained no penicillin and the children in the other school were given the same dentifrice to which 500 units of penicillin per Gm. of tooth powder had been added. The children, who came from similar economic and racial backgrounds, were instructed to use the powders at home also, morning and evening. Although there was no way of ascertaining how regularly they did so, there is reason to believe that they cooperated. The daily use of penicillin in oral hygiene may exert some influence on the penicillin sensitivity of the bacterial flora of the throat.

Therefore, in order to study penicillin sensitivity of streptococci and staphylococci isolated from the throats of both groups of children, the following program was followed: Throat swabs were taken from 190 children in the penicillin school and 154 children in the control school in the fall of 1949, after they had been brushing their teeth with the respective dentifrices for approximately two years.

In preliminary trials for the isolation of streptococci and staphylococci, throat swabs of the children were streaked directly on to blood agar plates. This method was found to be less sensitive and required more time than inserting the swab directly into broth for preliminary incubation prior to plating. Therefore, the following procedure was used: The throat swabs were planted directly into a sterile tube containing 2 ml. of brain heart infusion broth (Difco). After incubation for 2 to 4 hours at 37° C., a single blood agar plate was streaked to isolate colonies.

Plates were read after 24 hours' incubation, with the exception of a few which had too sparse a growth to permit adequate picking of colonies and required incubation for 48 hours. Selected colonies were picked into brain heart infusion broth, grown for 18 to 24 hours and, after restreaking onto blood agar, were gram stained to insure purity. In many instances, more than one colony was picked, particularly where more than one type of organism was recognized.

Tube dilution method for penicillin (penicillin G) sensitivities was run immediately. Those colonies which had to be kept for more than 24 hours were streaked on to blood agar plates and refrigerated until used. The dilu-

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tions of penicillin were taken out to 0.0011 units per ml. for every organism tested. *Staphylococcus aureus* (Oxford stain) was the organism used for control and was sensitive to 0.078 units of penicillin per ml. The serial tube dilution method was used, in which 0.5 c.c. of 1:1000 dilution of a 24-hour broth culture was added to 0.5 c.c. of decreasing concentrations of penicillin, starting with 10 units per ml. followed by twofold dilutions, that is, 10, 5, 2.5, 1.25, 0.62, 0.31, 0.156, 0.078, 0.039, 0.019, 0.009, 0.005, 0.0022, 0.0011, and so forth. The tube containing the least amount of penicillin and showing complete inhibition was selected as that amount to which the organism was sensitive.

TABLE I

BACTERIAL FLORA ISOLATED FROM THROATS OF CHILDREN IN CONTROL AND PENICILLIN SCHOOLS

TYPE OF BACTERIA	PERCENTAGE OF SUBJECTS HAVING ORGANISMS PRESENT: AS PREDOMINANT (%)	AS SECONDARY* (%)
Control School		
A. Nonhemolytic streptococci	1.3	6.9
B. Pneumococci	30.0	11.0
C. Beta hemolytic streptococci	10.0	4.1
D. Alpha hemolytic streptococci	38.7	9.6
E. Nonhemolytic *Staphylococcus aureus*	14.0	34.2
F. Hemolytic *Staphylococcus aureus*	0.7	0.0
G. *Staphylococcus albus*	0.0	27.4
H. Alpha prime streptococci	0.0	2.7
I. *Neisseria catarrhalis* (gram-negative diplococci)	4.7	4.1
J. Hemophilus group, gram-negative bacilli, diphtheroids, yeasts, etc.	0.7	0.0
Total = 150. No growth initially on 4	100.1	100.0
Penicillin School		
A. Nonhemolytic streptococci	1.1	3.0
B. Pneumococci	38.1	6.0
C. Beta hemolytic streptococci	6.9	4.5
D. Alpha hemolytic streptococci	39.7	39.1
E. Nonhemolytic *Staphylococcus aureus*	7.9	10.5
F. Hemolytic *Staphylococcus aureus*	0.0	0.8
G. *Staphylococcus albus*	0.5	22.6
H. Alpha prime streptococci	0.0	1.5
I. Neisseria catarrhalis (gram-negative diplococci)	3.2	6.7
J. Hemophilus group, gram-negative bacilli, diphtheroids, yeasts, etc.	2.6	5.3
Total = 189. No growth initially on 1	100.0	100.0

*The numbers in this column refer to the frequency in which the organism was found secondary to the predominant organism.

RESULTS

From 293 of the 344 children, one or more organisms were isolated. From these, 115 penicillin sensitivities were run from the control group and 144 from the test group.

In 25 children, a single organism initially isolated (streptococcus) failed to survive on transfer. In addition, from 21 patients, one of the organisms isolated (streptococcus or staphylococcus) failed to survive initial transfer, and from 5 patients, no organisms could be isolated, 4 from the group receiving penicillin and 1 from control group.

TABLE II
RESISTANCE OF ORAL BACTERIA TO PENICILLIN

CONCENTRATION (UNITS)	ALPHA HEMOLYTIC STREPTOCOCCI				BETA HEMOLYTIC STREPTOCOCCI				NONHEMOLYTIC STREPTOCOCCI				ALPHA PRIME STREPTOCOCCI				NONHEMOLYTIC STAPHYLOCOCCUS AUREUS				HEMOLYTIC STAPHYLOCOCCUS AUREUS			
	CONTROL		PENICILLIN		CONTROL		PENICILLIN		CONTROL		PENICILLIN		CONTROL		PENICILLIN		CONTROL		PENICILLIN		CONTROL		PENICILLIN	
	NO.*	%*	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
10.0									1	0.65														
5.0																	1	0.65						
2.5			2	1.05																				
1.25	2	1.30	3	1.58													2	1.30						
0.62			8	4.21											1	0.53	3	1.95						
0.31	1	0.65	11	5.79			1	0.53			1	0.53	1	0.65			2	1.30	6	3.16	1	0.65		
0.156	5	3.25	18	9.47			1	0.53	1	0.65	1	0.53					5	3.25	6	3.16				
0.078	4	2.60	22	11.58	1	0.65	2	1.05	1	0.65	1	0.53					15	9.74	7	3.68				
0.039	10	6.49	13	6.84	2	1.30	3	1.58	1	0.65					1	0.53	2	1.30	6	3.16				
0.019	13	8.44	8	4.21							2	1.05												
0.009	5	3.25	9	4.74	2	1.30	1	0.53	2	1.30			1	0.65	1	0.53	2	1.30						
0.005	6	3.90	4	2.11	3	1.95	2	1.05									3	1.95						
0.0022	1	0.65			1	0.65											5	3.25						
0.0011			4	2.11	2	1.30	1	0.53									5	3.25						
<0.0011					3	1.95																		
Total	47	30.32	102	53.68	14	9.09	11	5.79	6	3.89	5	2.63	2	1.30	3	1.58	45	29.22	25	13.16	1	0.65		
Average sensitivity	0.0982		0.2197		0.0141		0.0690		1.7152		0.1164		0.1595		0.2227		0.2679		0.1430		0.310			
Median sensitivity	0.019		0.078		0.005		0.039		0.585		0.078		0.1595		0.039		0.078		0.078		0.310			

*Numbers and percentages indicate that organism was found sensitive to penicillin at concentration tested. Percentages are based on number of children in each school from whom throat cultures were taken: control, 154; penicillin, 190.

From the experimental group, as well as the control group, alpha hemolytic streptococcus was the predominating organism most frequently isolated, with pneumococcus a close second; 40 and 38 per cent versus 39 and 30 per cent, respectively. Nonhemolytic *Staphylococcus aureus* and beta hemolytic streptococcus comprised numbers three and four respectively in frequency. In the control group nonhemolytic *Staphylococcus aureus* was most frequently the other organism, while in the experimental group, the other organism most frequently isolated was alpha hemolytic streptococcus. This can be seen in Table I.

Results of penicillin sensitivity tests on bacteria isolated are shown in Table II. From this table, it can be seen that in both groups the sensitivity of the organisms was well within the range normally found for these organisms, even though some were found to be sensitive to 2.5 units, especially in the alpha hemolytic streptococci group. This is a sensitivity range which is still considered acceptable for treatment with penicillin.[2] The two cases in which the organisms were sensitive to 5 and 10 units were found in the control school. Regardless of in vitro sensitivity results, most workers consider an organism sensitive to 10 units or less of penicillin to be treatable. However, it is interesting to note that the average sensitivity value was higher in the treated group than in the control group for streptococci, while the reverse was true with staphylococci which were isolated.

CONCLUSION

The results from this study are sufficiently comparable in both the test and the control groups to conclude that penicillin did not induce an acquired resistance in the groups of streptococci and staphylococci isolated from the throats.

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A STUDY OF STREPTOCOCCUS SALIVARIUS AND ITS RELATIONSHIP TO THE DENTAL CARIES PROCESS

FREDERIC R. SHIERE, D.D.S., M.S.,* CARL E. GEORGI, PH.D., AND RALPH L. IRELAND, D.D.S., M.S.

Department of Pedodontics, College of Dentistry and Department of Bacteriology University of Nebraska, Lincoln, Neb.

THE purpose of this investigation concerns (1) the role of *Streptococcus salivarius*, a normal inhabitant of the mouth and a potent acid producer in vitro, as a factor in initiating dental caries, and (2) its use as a diagnostic aid to determine caries susceptibility or caries immunity.

The association of other acidogenic bacteria with dental caries, principally the lactic acid-producing rods, has been extensively investigated. However, the streptococcal acidophilic flora of the oral cavity has not been studied to any such extent. In addition, very few attempts have been made to distinguish *Str. salivarius* from other viridans or alpha hemolytic types of streptococci that occur in the mouth.

No previous investigation in vitro has shown the effect of sodium fluoride on the teeth in the presence of *Str. salivarius*. Neither has any other investigation in vitro demonstrated quantity of sugar consumed nor the amount of acid produced by these microorganisms in the presence of tooth substance.

LITERATURE

Several investigators have considered that streptococci might be involved in the caries process. J. Leon Williams (1897)[1] stated that in marked decay of enamel streptococci far outnumbered all other forms of microorganisms. Rettger (1934)[2] demonstrated the numerical importance of high acid-producing streptococci as compared to lactobacilli in carious and noncarious mouths. The former were present in greater numbers per cubic centimeter of saliva than the latter. He stated that the streptococci would appear to be more important than the lactobacilli. Hammond and Tunnicliff (1940)[3] incubated 16 sterile teeth in a 1 per cent dextrose broth and inoculated this broth with *Streptococcus viridans* (smooth colony). Fresh broth was added weekly to maintain a pH of 4.2. After 14 days, the enamel was etched in a manner which the authors stated was similar to early natural caries. Bibby, Volker, and Van Kesteren (1942)[4] found that streptococci produced acid more rapidly than any other bacteria tested. The discovery that certain organisms produced more acid than others in the presence of enamel and dentin in vitro suggested that certain bacteria were especially well adapted to produce acid in the presence of tooth substance. Thus, it appeared that certain streptococci were important in pro-

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*Present address: Tufts College Dental School Boston, Mass.

ducing decalcification of the teeth. The direct relationship between the acid formed and the amount of enamel and dentin lost indicated that this loss was entirely the result of the action of bacterial acids. Parsons and Frobisher (1944)[5] concluded that the acidogenic cocci seemed to be of greater significance than lactobacilli in maintaining an acid reaction in the crypts of the teeth of rats, especially those fed on a high carbohydrate diet.

The first investigators to describe *Str. salivarius* adequately were Andrewes and Horder (1906)[6] who characterized it as a normal constituent of saliva, occurring in short chains, rendering broth turbid, failing to liquefy gelatin at 20° C., clotting milk, and capable of reducing neutral red. They found that it consistently fermented sucrose, lactose, and raffinose, rarely fermented inulin and never fermented mannitol. Safford, Sherman, and Hodge (1937)[7] isolated 322 cultures of nonhemolytic streptococci from the human throat. *Str. salivarius* prevailed in 290 of the isolates studied. The remaining 32 varied somewhat, but not enough to be considered separate species. They described *Str. salivarius* as follows: It occurred in short chains; maximum temperature of growth at 45° C.; did not survive in milk when heated to 60° C. for 30 minutes; produced a final pH of 4.4 to 4.0 in glucose broth; acidulated and curdled litmus milk; did not hydrolyze sodium hippurate; did not produce ammonia from peptone; did not hydrolyze starch; and did not liquefy gelatin. It fermented glucose, maltose, lactose, and sucrose; was nonpathogenic; was readily lost with infrequent transfer, and its human habitat was the mouth and throat.

Niven, Smiley, and Sherman (1941)[8] found that many cultures of *Str. salivarius* produced copious amounts of polysaccharide from sucrose when grown on solid media, resulting in the formation of large mucoid colonies. These mucoid colonies were also produced from raffinose, but not from the other carbohydrates. Smiley, Niven, and Sherman (1942)[9] stated that *Str. salivarius* had a marked ability to synthesize a large amount of soluble levan from sucrose, and that this property was limited to *Str. salivarius*. Smith and Sherman (1942)[10] made 286 tests with 158 cultures of streptococci to determine their ability to produce lactic acid. *Str. salivarius* was capable of fermenting 93.6 per cent of the glucose in the medium and this was the highest producer of lactic acid of the viridans group.

METHODS AND MATERIALS

I. *Quantitative*

A quantitative study of the incidence of *Str. salivarius* in the oral cavity was the first objective. In this experiment, saliva samples were collected from children and adults regardless of the presence or absence of carious lesions. The donors were arbitrarily divided into two groups for comparison. The first group was selected at random from students and associates while the second group was selected from the Nebraska State Home for Children.

A small, wrapped piece of paraffin was given to the patient who was instructed to chew the paraffin for 3 minutes and to collect, aseptically, approximately 3 ml. of saliva in a small, wide-mouthed sterile bottle. The patient was requested to carry out this procedure in the morning before brushing the teeth,

rinsing the mouth, or eating. At this time, the maximum bacterial population could be expected to be found in samples of saliva as demonstrated by Slanetz and Brown.[11] The saliva samples were plated immediately whenever possible. In the event that this was impossible, the samples were placed in a refrigerator and plated not longer than 3 hours after collection.

A portion of the original sample of saliva was used for electrometric pH determinations, the remainder to estimate the bacterial population. One ml. of saliva was transferred to water blanks of normal physiologic saline and triplicate plates were poured at dilutions of 1:100,000 and 1:1,000,000. An uninoculated control plate was simultaneously poured, in order to determine whether the medium and glassware were sterile.

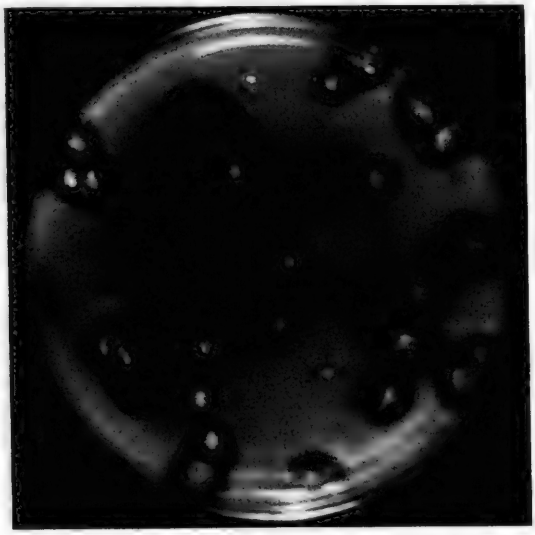


Fig. 1.—Surface and deep colonies of *Str. salivarius*, from a saliva specimen grown on the medium of Niven, Smiley, and Sherman.[8] Halos are the result of neutralization of $CaCO_3$ by lactic acid produced by the bacteria.

The medium used for this work was the same as that suggested by Niven, Smiley, and Sherman[8] for the growth of *Str. salivarius*. On this substrate the organism produces a large amount of polysaccharide from sucrose and forms large, readily detectable mucoid colonies. Its composition follows:

Tryptone (Bacto)	10.0 Gm.
Yeast extract (Bacto)	5.0 Gm.
Gelatin (Bacto)	50.0 Gm.
Agar (Bacto)	15.0 Gm.
Sucrose (technical)	50.0 Gm.
Calcium carbonate (U.S.P.)	5.0 Gm.
Distilled water q. s.	1000.0 ml.

Approximately 15 ml. of this medium was used per Petri dish. The latter were incubated for 48 hours at 37° C. after which colony counts were made.

The medium proved quite selective. The colonies with the characteristic halo (Fig. 1) were counted as typical of *Str. salivarius* regardless of their smooth or rough surface topography.

The teeth of the patients from whom the saliva was obtained were examined clinically to detect the presence or absence of any carious lesions. The mouth mirror and explorer were used primarily for this purpose and whenever possible bite-wing radiographs were also employed. The D.M.F. (decayed, missing, and filled teeth) was noted and recorded. All areas on the occlusal, buccal, labial, or lingual surfaces of the teeth (pits and fissures) in which the explorer sank beneath the point of entrance were considered precarious areas and recorded as carious. Etchings on the mesial and distal surfaces of the teeth, which were disclosed by means of roentgenograms, were also recorded as carious even though the etching did not appear to have penetrated to the dentin.

II. *Qualitative*

The second objective was to examine representative colonies observed on the selective medium in order to establish and confirm their identity as *Str. salivarius*. Thirty-eight of the organisms which were isolated from the saliva were used in this study. All of these organisms produced the mucoid colonies on the selective medium which was typical of *Str. salivarius*.

The basal medium used in this study contained:

Peptone (Bacto)	5.0 Gm.
Beef extract (Bacto)	3.0 Gm.
Yeast extract (Bacto)	5.0 Gm.
Carbohydrate	10.0 Gm.
Bromcresol purple	1.0 ml.
Distilled water q. s.	1000.0 ml.

A specified carbohydrate was added to this basal medium for use in the fermentation tests. The carbohydrates were made in 10 per cent solutions and added to the sterile basal medium to yield a final concentration of 1 per cent. The indicator, bromcresol purple, was 1.6 per cent alcoholic solution, used at the rate of 1.0 ml. per 1000.0 ml. The 10 per cent solutions of carbohydrates (lactose, glucose, sucrose, mannitol, and raffinose) were autoclaved, while salicin was sterilized by filtration. The final pH produced by each strain was determined on both glucose and sucrose broth.

The identity of each culture was confirmed by gram staining before being studied. They appeared gram-positive and the cells occurred in short chains.

The isolates for use in the in vitro studies were maintained in a broth recommended by Niven:[12]

Tryptone (Bacto)	10.0 Gm.
Glucose (C.P.)	1.0 Gm.
Dipotassium phosphate (C.P.)	2.0 Gm.
Calcium carbonate (U.S.P.)	3.0 Gm.
Distilled water q.s.	1000.0 ml.

Str. salivarius was incubated for 48 hours at 37° C. in this broth. The microorganisms were transferred to solid stock medium of the same composition

as above, but with 1.5 per cent agar added. The cultures were then transferred every 3 weeks to maintain viability.

III. *Studies in vitro*

The third and final objective was to make a series of in vitro studies to determine, if possible: (1) the effect of *Str. salivarius* on the enamel of freshly extracted teeth, (2) the effect of *Str. salivarius* on the teeth in the presence of 1 ppm* of sodium fluoride, and (3) to demonstrate the amount of sugar utilized and the amount of acid produced by these microorganisms in the presence of tooth substance.

In order to conduct the experiment, several freshly extracted teeth were collected. They were cleaned of all adhering substances and were stored in the refrigerator in a flask containing distilled water.

The teeth were sterilized, in individual tubes containing a small amount of water, by autoclaving for 20 minutes at 15 pounds' pressure. The teeth were removed and under ultraviolet light were covered with a Vinylite solution, and the latter then allowed to dry. A small area of Vinylite on the labial surface of each tooth was then removed aseptically, thus permitting contact of a portion of the tooth surface with the microorganisms or their products of metabolism when introduced in culture media.

In order to test the sterility at this point, the teeth were placed in broth which contained:

Tryptone (Bacto)	10.0 Gm.
Yeast extract (Bacto)	5.0 Gm.
Dipotassium phosphate (C.P.)	2.0 Gm.
Glucose (C.P.)	10.0 Gm.
or	
Sucrose (technical)	10.0 Gm.
Distilled water q. s.	1000.0 ml.
pH-7.0	

One-half, or approximately 30 tubes, contained glucose as the carbohydrate, while the remainder (30 tubes) contained sucrose. The tubes were incubated for 48 hours at 37° C. to check the sterility of the teeth. The few tubes which showed contamination were eliminated. After the 48 hours' incubation period, the remaining tubes, containing the sterile teeth, were inoculated with cultures of *Str. salivarius* and incubated at 37° C.

As these organisms are not capable of tolerating the high concentrations of acid produced by their metabolism of glucose and sucrose, the teeth were aseptically transferred weekly to a tube of fresh medium. At the end of each 2-week period, 6 tubes were removed and the teeth were examined for etchings of the enamel surface. The final pH was also determined. This procedure was continued for a period of 2 months.

The use of sodium fluoride as an inhibitory agent in the control of dental caries and its role in the glycolytic cycle are well known. Thus it was decided to determine what effect its presence has on *Str. salivarius* by using sodium

*Meaning = parts per million.

fluoride in a few of these same tubes. One ml. containing 1 ppm sodium fluoride was then placed in 2 of the tubes containing glucose and in 2 of the tubes containing sucrose.

In determining the quantity of sugar utilized by these organisms, the reducing sugar analysis of Stiles, Peterson, and Fred[13] was used.

EXPERIMENTAL RESULTS

I. *Quantitative*

A practical importance of *Str. salivarius* would be demonstrated if it were associated with dental caries. The ideal method of making such a demonstration would be to correlate the incidence of dental caries during the previous year with the *Str. salivarius* count of saliva. This information is not available for the group of subjects included in this test. It was considered that some clue to such association might be obtained if the *Str. salivarius* count were related to the number of open cavities, which might be of sufficient value yet a less perfect indication of caries activity.

There were 2 groups of subjects for which such a correlation could be tested, 70 cases picked at random, and 44 cases from the Nebraska State Home for Children, as shown in Table I. The latter were receiving continuous dental treatment which would prevent backlogs of untreated cavities from developing, and the incidence of decayed teeth in this group would constitute our best index of dental caries rate.

TABLE I
CORRELATION COEFFICIENT: D.M.F.: D.M.F. VS. STR. SALIVARIUS

	MALE	FEMALE	D.*	M.†	F.‡	D.M.F.§
			Group 1			
Adults	35	23	0.140	0.038	0.192	0.233
Children	4	8				
			Group 2			
Children	20	24	0.426	0.297	0.150	0.394

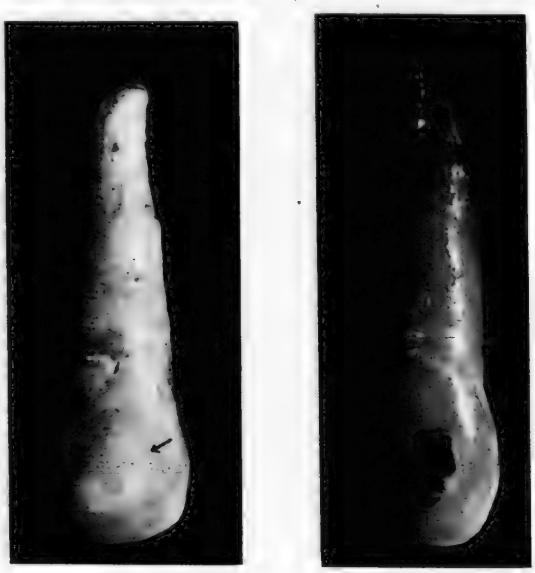
*D. = decayed teeth.
†M. = missing teeth.
‡F. = filled teeth.
§D.M.F. = decayed, missing and filled teeth.

Correlation coefficients are also presented in Table I between the decayed, missing, and filled teeth, the total D.M.F., and the *Str. salivarius* counts at 1:1,000,000 dilution. The correlation coefficient between decayed teeth and the *Str. salivarius* counts for the 44 cases is + 0.43 and is statistically significant at the 1 per cent level.[14] There is also significant correlation with missing teeth and D.M.F. Among the 70 cases where backlogs of unfilled cavities might obscure the correlation, the coefficients are less, and there is only a suggestion of a relationship. These findings give encouragement to the hypothesis that *Str. salivarius* is associated with dental caries.

The pH of the stimulated saliva was essentially alkaline. Seven saliva samples were in the vicinity of pH 8.0 or slightly higher, while only two samples were below pH 6.0; the average was pH 7.6. Three of the samples were insufficient in quantity to allow for electrometric pH measurement.

II. *Qualitative*

The "typical" colony of *Str. salivarius* synthesizes a large amount of levan when grown on the sucrose medium of Niven, Smiley and Sherman.[8] This was observed by the formation of large mucoid colonies. Smiley, Niven, and Sherman[9] stated that no other known streptococcus possesses this property. Thus, the isolation from saliva of these bacteria appearing as mucoid colonies on the medium suggested by Niven was a relatively simple matter (Fig. 1.) Colonies of *Str. salivarius* were "fished" from this medium for identification by biochemical reactions. A total of 38 isolates were thus obtained and all of the organisms fermented glucose and sucrose. Two of the strains failed to ferment raffinose, while 1 failed to ferment salicin. None of the organisms produced acid from mannitol. While *Str. salivarius* will almost always ferment lactose, 6 isolates failed to do so. Strains of nonlactose-fermenting streptococci isolated



A. *B.*

Fig. 2.—Changes on the surface of the enamel after immersion for 1 month in carbohydrate broth inoculated with *Str. salivarius*. *A*, Nonpigmented. *B*, Pigmented.

from the human throat have been reported by Arnold.[15] Thirty-two cultures, however, gave fermentation reactions similar to those described by Andrewes and Horder.[6] When final pH determinations were made, the broth cultures containing sucrose generally attained a lower pH range (3.9 to 4.8) than those with glucose (4.2 to 5.0.) Of the 38 cultures tested, 26 gave a lower pH reading in the sucrose broth than in the glucose broth. Each isolated culture was gram-positive. The isolates obtained from the solid medium appeared as cocci in short chains, while those obtained from the broth were in longer chains.

III. *Studies in vitro*

At the conclusion of the first 2-week period, in which a small area of the extracted teeth had been exposed to the action of *Str. salivarius* and its metabolic

products, etching of the enamel surface was observed. However, the area was much more clearly outlined at the end of 1- to 2-month periods. This phenomenon may be observed in Fig. 2, *A* and *B*. The gross appearance of the tooth structure shows a white area in which the interprismatic substance of the tooth has been attacked. A brown stain surrounded by a white area was also observed on many of the teeth (Fig. 2, *B*.)

Over a 5-week period, 5 strains of *Str. salivarius* were transferred once a week into a series of glucose broth tubes and a parallel series of sucrose broth tubes, each containing a single tooth. The pH was measured at the end of each week's fermentation and found to drop from pH 7.0 to pH 4.2 to 4.8. The latter pH range is well within that required for decalcification of the enamel.

A limited study was made of the effect of sodium fluoride on *Str. salivarius*. No growth of the organism occurred after exposure to 1 ppm for 48 hours. The presence of sodium fluoride thus acts as an inhibitory agent to the growth of *Str. salivarius*. The role of sodium fluoride as an inhibitor in carbohydrate metabolism is well established.

TABLE II

CARBOHYDRATE UTILIZATION

TIME (HRS.)	GLUCOSE REMAINING %	SUCROSE REMAINING
6	0.87	0.89
12	0.79	0.85
18	0.73	0.72
24	0.67	0.67
48	0.47	0.46
Uninoculated control	0.87	0.90

TOTAL ACIDITY

TIME (HRS.)	% LACTIC ACID PRODUCED FROM GLUCOSE	SUCROSE
48	0.49	0.51
48	0.50	0.45

The results of carbohydrate fermentation of *Str. salivarius* may be observed in Table II. At the end of 6 hours very little of either glucose or sucrose had been consumed. At the end of 18 hours approximately 25 per cent of the sugar had been utilized, while at the end of 48 hours approximately 50 per cent had been consumed. Also, at the end of 48 hours approximately 0.5 per cent acid (calculated as lactic) was produced. This was enough to lower the pH below 5.0 which is capable of decalcifying enamel.

DISCUSSION

The presence of *Str. salivarius* as a normal constituent of saliva was confirmed. From the many saliva samples tested, only 2 contained no colonies of *Str. salivarius*. The organism was found to be gram-positive and a coccus growing in chains. On a solid medium the chains were comparatively shorter than when grown in a broth medium. *Str. salivarius* fermented glucose, sucrose, lac-

tose, raffinose, and salicin, but never mannitol. It grew well when incubated at 37° C. on the selective medium provided for it. It was necessary to transfer the organism approximately every 3 weeks to maintain viability. It was also capable of producing acid in carbohydrate medium. On plating medium, *Str. salivarius* was capable of producing large mucoid colonies resulting from the production of a polysaccharide gum. This gum is a levan which is produced from the sucrose of the medium and which possesses the available terminal d-fructo-furanose residue which is required for levan production. As *Str. salivarius* is a homofermentative lactic acid producer, in which the end product is solely lactic acid, it can be assumed that the acid produced was lactic.

For further proof of the identity of *Str. salivarius,* Cultures S20B and S30D were obtained from Cornell University and the American Type Culture Collection, respectively. Tests on these organisms were conducted simultaneously with isolates from saliva of patients reported in this investigation. The results with S20B and S30D were identical with isolates made during this study.

Favorable statistical evidence showed that in a selected group of patients there was a definite correlation between decayed and missing teeth and D.M.F. with *Str. salivarius* at the 1:1,000,000 dilution. With this available evidence, it is possible that *Str. salivarius* is associated with dental caries.

Following the immersion of the extracted teeth in the inoculated sugar broth, a change in the tooth structure is readily demonstrated. Kronfeld[16] expressed the opinion that the first visible evidence of caries on an intact surface is a change in the optical properties of the enamel. Pigment may or may not be present. The enamel structure consists of the rods and the cementing substance between these rods. The interprismatic cementing substance consists solely of inorganic salts (Ca, P, CO_3,Mg) which are readily soluble in dilute acids. It would seem then that the acids formed from the carbohydrate on which *Str. salivarius* was cultivated has initiated in vitro caries of the enamel which is similar to early natural caries in the mouth.

Sodium fluoride acts as an inhibitory agent in the presence of *Str. salivarius.* Fosdick[17] stated that any inhibitor that would block any one of the reactions in carbohydrate fermentation would prevent the formation of lactic acid. The enzyme inhibitor must also be present in the mouth when the acids are being formed. It is known that the fluoride ion can be maintained in susceptible areas of the mouth for long periods of time. Thus, it can be seen that sodium fluoride could inhibit the action of the acids formed by *Str. salivarius* and thus reduce the formation of carious lesions.

The ability of *Str. salivarius* to utilize appreciable quantities of sugar, with the resultant production of acid in the pH range suitable for decalcification of the enamel, shows that this organism must be considered as a possible etiologic agent of dental caries. Carious lesions occur in certain susceptible areas on the teeth which are not readily reached by the free-flowing saliva nor by mechanical cleansing agents. Thus, the accumulation of debris in these areas and in the presence of *Str. salivarius* could produce a carious lesion.

SUMMARY

1. *Str. salivarius* is a normal inhabitant of the saliva.

2. The relationship of decayed and missing teeth, and the D.M.F. to caries activity, has been demonstrated.

3. Carbohydrate degradation of *Str. salivarius* lowers the pH between 4.0 to 5.0 which is sufficient for decalcification of the enamel.

4. Etching of the intact enamel surface has been demonstrated in vitro.

5. The action of *Str. salivarius* is inhibited by the presence of 1 ppm of sodium fluoride.

6. *Str. salivarius* can readily convert some common fermentable sugars to lactic acid.

7. *Str. salivarius* may be considered as a potential etiologic agent of dental caries.

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THE ACIDITY OF SALIVA AFTER INGESTION OF CARBOHYDRATE- AND ACID-CONTAINING SUBSTANCES

H. W. HAGGARD, M.D., AND LEON A. GREENBERG, PH.D.

From the Laboratory of Applied Physiology, Yale University, New Haven, Conn.

THE Bureau of Public Relations of the American Dental Association has stated that there is little doubt that the direct cause of tooth decay is the breakdown of carbohydrate foodstuffs on the surface of the teeth, forming acids,[1] and that it is now widely believed that decay of the enamel is caused by acids produced in fermentation of sugars.[2] In studying a factor which might have a bearing on this process, we have investigated and previously reported data on the magnitude and duration of concentrations of carbohydrate in the saliva after ingestion of a variety of common carbohydrate-containing substances.[3]

It is now generally accepted by dental research workers that this decalcifying action of acid resulting from carbohydrate in the mouth is not related to acidity of the saliva but is due to isolated areas of high acidity on the surfaces of the teeth which are protected from the diluting and neutralizing action of the saliva. However, a number of studies have been recently reported in which teeth were immersed in acidulated liquids in vitro,[4] or in which acidulated liquids were fed to animals[5-11] with resulting erosion of the teeth. Although several of these reports have warned that the animal experiments do not justify any conclusions as to the effects on the teeth of acid beverage ingestion in man,[10, 11] it could be inferred from most of them that the normal human ingestion of acid fruit juices or acidulated soft drinks may damage the teeth. With this possibility in mind we have studied, in the present investigation, the extent and duration of acidity occurring in the mouth in normal human subjects following the ingestion of a variety of acid as well as nonacid foods.

The substances studied were a mixed meal, fresh orange juice, grape juice, a bottled cola beverage, tomato juice, ice cream, caramel candy, and crackers. The subjects were 16 normal healthy adults with no active caries. Each of the subjects was tested with each of the foods.

Before taking any of the test substances, the teeth and mouth of each subject were thoroughly cleansed with a brush and by rinsing. A preliminary sample of saliva was then taken and its acidity determined by means of the Beckman glass electrode pH meter. After the test substances were taken, samples of saliva were collected at 5, 10, 15, 30, 60, and 90 minutes. The pH of each of these samples was also determined by the glass electrode. The data shown in Table I and Figs. 1 and 2 represent the mean values of the tests on separate subjects with each test substance. The pH of the acid substances used are also shown in Table I.

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As may be seen from these results the nonacid substances tested resulted in no significant changes in the acidity of the saliva for a period of one and one-half hours after their ingestion. The acid beverages caused a decrease in

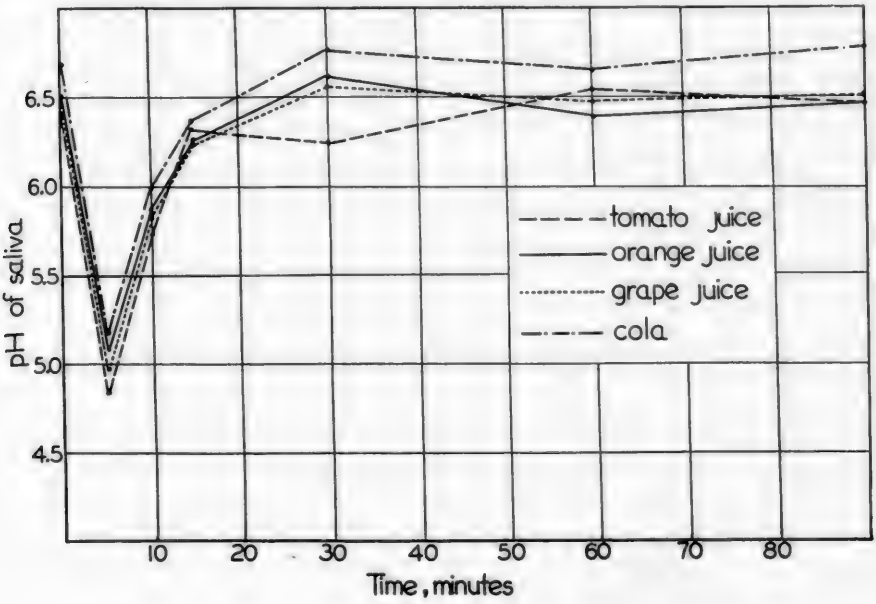

Fig. 1.

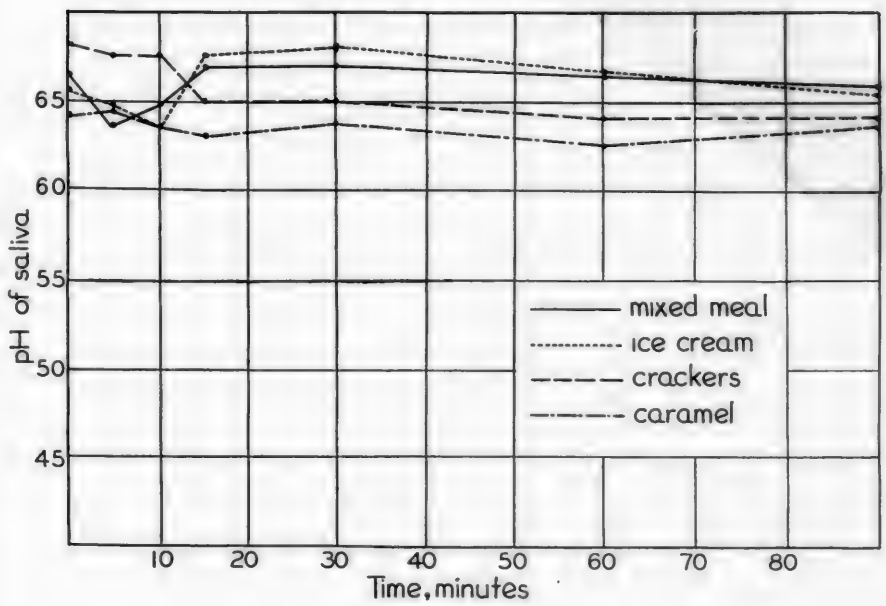

Fig. 2.

the pH of the saliva of short duration. The effectiveness of the continuously secreted saliva in buffering and washing away this acid from the mouth is apparent in the rapidity with which the reaction of the saliva returns to its essentially initial level. At no time after ingestion of the acidulated beverages did the pH of the saliva reach the values of 3.5 to 4.5, given by McClelland[12] as the erosive level. The highest acidities were reached with the commonly used fruit juices. The duration of acidity in the mouth greater than pH values of 5.0 to 5.5, described by Stephan[13] as the "critical decalcifying level," was so brief as to render damage to the teeth unlikely. In this conclusion we are in agreement with those of Pincus,[14] Stephan,[13] and Wallace[15] that a decalcifying concentration of acid does not persist in the saliva, that there is no evidence to indicate that the ingestion of acid foods has any etiologic relationship with caries, and that there is no evidence that foods which are acid are harmful to the teeth.

TABLE I

SUBSTANCE	pH OF SUBSTANCE	pH OF SALIVA MINUTES AFTER INGESTION						
		PRELIM.	5	10	15	30	60	90
Mixed meal		6.64	6.34	6.48	6.70	6.70	6.62	6.56
Crackers		6.80	6.76	6.76	6.50	6.50	6.40	6.40
Ice cream		6.54	6.48	6.34	6.76	6.80	6.64	6.56
Caramel candy		6.40	6.42	6.34	6.30	6.38	6.24	6.36
Orange juice	3.49	6.50	5.08	5.86	6.26	6.62	6.38	6.48
Tomato juice	4.22	6.48	4.84	5.74	6.30	6.24	6.52	6.48
Grape juice	3.04	6.46	4.92	5.32	6.24	6.54	6.48	6.50
Cola beverage	2.64	6.70	5.18	5.98	6.34	6.76	6.68	6.84

If it is true that there are, in fact, persistent areas of carbohydrate and corrosive acidity on sheltered surfaces of the teeth and under plaques which are protected from dilution and neutralization by the saliva, it would be expected that this protection would also operate against further acidification by the brief tides of acidity occurring in the saliva after ingestion of the acidulated beverages. Added to this is the recent observation by Stralfors[16] that the dentobacterial plaque under which caries begins is even a better buffer than saliva itself.

SUMMARY

1. The ingestion of carbohydrate-containing substances causes no significant acidity of the saliva.

2. The ingestion of acidulated beverages such as common fruit juices or soft drinks results in acidity of the saliva for a short period of time.

3. A corrosive action, so as to cause dental caries, either on the exposed surfaces of the teeth or in sheltered areas, by the acidity of fruit juices or soft drinks, appears unlikely in the normal consumption of these beverages.

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THE EFFECTS OF TEMPERATURE CHANGES ON SALIVARY AMYLASE ACTIVITY

LEON H. SCHNEYER

Dental Department, Montefiore Hospital, New York, N. Y.

THIS investigation is concerned with delineating the effects of changes in temperature on the action of salivary amylase. Particular attention is paid to the reversibility of the denaturing effects of high temperatures and to the mechanism of the antidenaturant action of the substrate.

Tokuoka (1939),[1] working with alpha amylases from various sources, but not from saliva, found less than 5 per cent amylase regeneration after heating for 15 minutes at a temperature well above the optimum and then cooling to room temperature. He reported protection of the amylase against heat inactivation by calcium salts, starch, and proteins. Tokuoka found no protection by NaCl.

The data to be presented here indicates that salivary amylase, like trypsin,[2] luciferase,[3, 6] and sucrase,[4] undergoes a true reversible denaturation. Further, the data confirms the antidenaturant action of the substrate and indicates that this action is mediated through an effect on the reversible denaturation process. In addition, the irreversible denaturation process is slowed by NaCl.

Salivary amylase, like other enzymes, shows a characteristic change in activity with a change in temperature. In general, enzyme activity increases with a rise in temperature up to the optimum temperature, and thereafter, as the temperature is raised, the activity seems to decrease. This is because every enzymic reaction consists actually of two antagonistic reactions. One of these is the enzyme-substrate reaction; the other is the enzyme denaturation reaction. At temperatures below the optimum the enzyme-substrate reaction is dominant, and a rise in temperature within this range increases the rate of the process. At temperatures above the optimum, the denaturation reaction is dominant, and a rise in temperature within this range further depletes the system of active native enzyme and hence apparently slows the enzyme-substrate reaction.

Denaturation of a protein involves breaking of chemical bonds within the molecular structure of the protein. Chemical groupings such as SH and OH which, in the native protein are held in tight combination and are not reactive to any great extent with externally added agents, after denaturation become free at the molecular surface and may be detected by chemical means. The catalytic activity, a function of the peculiarly individual structure of the protein molecule, is generally lost. In addition, the denaturation process is characterized by a very large heat of activation, that is, 50,000 calories or more, as would be expected if a number of chemical bonds were broken in the process.

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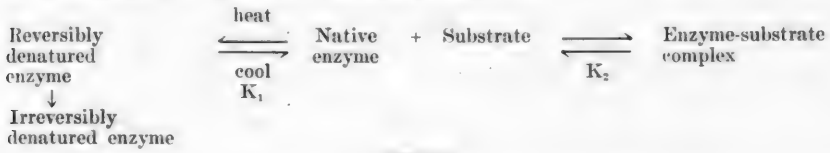
The thermal denaturation of many enzymes proceeds in two phases. The first is reversible by a reduction in temperature; the second is irreversible. The relationship between reversibly denatured enzyme, native enzyme, and irreversibly denatured enzyme may be schematized as follows, where K_1 is the equilibrium or rate constant for the reversible denaturation reaction and K_2 is the constant for the enzyme-substrate reaction:



METHOD

Freshly collected, mixed human saliva diluted with 0.25 per cent NaCl, usually 1:1000, comprised the enzyme solution. The determination of amylase activity was made by Somogyi's iodine method.[5] A constant temperature water bath was employed for all experiments.

All measurements are of time to reach an end point in starch digestion. The end point selected is the point of disappearance of the violet color, as suggested by Somogyi. The length of time to reach the end point of amylolysis bears an inverse relationship to the rate of the process. Since the end point represents a fixed point in the course of the digestion, the rate of amylolysis in an experimental solution relative to the rate in a control solution may be determined from the simple Newtonian rate formula

$$S = rt \qquad (1)$$

where S is distance to the end point; r is rate of the reaction (r_1 = rate of the control, and r_2 = rate of the experimental); and t is time to the end point (t_1 for the control time, t_2 for the experimental). Since $S_1 = S_2$

$$r_1t_1 = r_2t_2. \qquad (2)$$

If the rate of the control be set equal to 100 per cent,

$$r_2 = \frac{100\ t_1}{t_2}. \qquad (3)$$

Then, "r_2" represents the relative rate of the enzyme-substrate reaction under experimental conditions and expresses the relative activity of the enzyme.

Since a measurement only of relative activity was desired, and since a control was run for each experiment, the incubation time was selected to fit the conditions of the experiment, even though this time frequently fell outside of the limits of 8 to 20 minutes suggested by Somogyi for the digestion time.

RESULTS

The results of two experiments in which amylase activity was measured at selected temperature points between 7° and 58° C. are given in Fig. 1, *A*. Amylase activity is expressed in terms of per cent of activity at 50° C. which is set at 100 per cent, and the logs of these values are plotted against the reciprocal of the absolute temperature for each case. Temperature in degrees

centigrade is written in for ease of interpretation. This arrangement of the data facilitates the determination of the heat of activation or Arrhenius u value for the amylase-substrate reaction.

Reference to Fig. 1, *A* shows that the activity of the amylase increases rapidly as the temperature is raised from 7° to 50° C. Beyond 50° C. there is a rapid drop in activity. The optimum temperature for the activity of salivary amylase under these conditions (pH about 6.8, NaCl 0.25 per cent, mixed human saliva, final dilution 1:4000) is, therefore, 50° C.

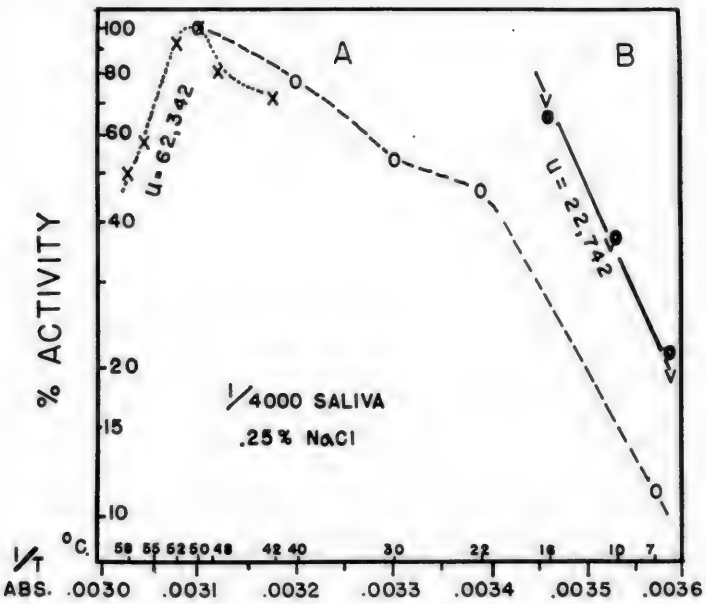


Fig. 1.—The relationship between amylase activity and temperature. Log per cent amylase activity is plotted against the reciprocal of the absolute temperature. The heat of activation for the enzyme-substrate reaction is calculated from the slope of the curve in *B* (two experiments). The heat of activation for the high temperature inactivation process is calculated from the slope of the linear portion of the curve beyond the optimum temperature (50° C.) in *A*.

A plateau in activity occurs before the optimum temperature is reached. The increase in activity with a rise in temperature in the range below 50° C. may be considered to result from the accelerating effect of temperature on the native amylase-starch reaction. This is the reaction governed by K_2.

The heat of activation or u value for the amylase-starch reaction may be calculated from the Arrhenius equation. The form of the equation used by Brown[6] to calculate u for bacterial luminescence is

$$\log \cdot \frac{k_2}{k_1} = \frac{u}{2} \; (1/T_1 - 1/T_2)$$

where k_1 and k_2 are the reaction velocities at temperatures T_1 and T_2. The u value for the amylase-starch reaction calculated from the low temperature data of the two experiments graphed in Fig. 1, *B* is 22,742 calories.

The appearance of the optimum and the decrease in activity as the temperature is raised beyond this point may be ascribed to the ascendency of a denaturation reaction. The heat of activation of the high temperature process is represented by the sum of the u values determined for the linear portions of the high and low temperature curves of Fig. 1. As stated by Brown[6] for bacterial luminescence, this method of determination "presupposes that reaction 2 (enzyme-substrate reaction) obeys the Arrhenius equation over the entire range and that reaction 1 (inactivation reaction) interferes to an extent sufficient to yield the positive linear slope at high temperatures." The u value for the inactivation process for salivary amylase, calculated on this basis, is 62,342 calories.

A				B			
°C.	MINUTES TO END PT.	% INHIBITED	% REVERS-IBILITY	°C.	MINUTES TO END PT.	% INHIBITED	% REVERS-IBILITY
44.	9.			50.	3.		
57.	24.	50.		60.	6.	50.	
AFTER 1.0 MIN. AT 57° C. *				AFTER .5MIN. AT 60°C. *			
44.	8.	0.	100.	50.	3.	0.	100.
C				D			
°C.	MINUTES TO END PT.	% INHIBITED	% REVERS-IBILITY	°C.	MINUTES TO END PT.	% INHIBITED	% REVERS-IBILITY
22.	6.5			22.	5.		
60.	.5			60.	3.		
AFTER 1.0 MIN. AT 60°C. *				AFTER 1.0 MIN. AT 60°C. #			
22.	5.0	0.	100.	22.	8.	38.	62.

* AMYLASE - .25% NaCl
\# AMYLASE - .05% NaCl

Table I.—The reversibility of the high temperature inactivation process.

Data presented in Table I indicates that one stage in this denaturation is reversible. One sample of enzyme solution with 0.25 per cent NaCl was kept one minute at 57° C., a temperature beyond the optimum and hence within the range where significant inactivation will occur, and then was placed in a water bath at 44° C., at which time the substrate was added. The rate of digestion was identical to that of an enzyme-substrate mixture kept only at 44° C. Had the denaturation which occurred at 57° C. proved irreversible, the activity after return to 44° C. would have been significantly less than that for a mixture kept only at 44° C.

Table II shows the results of experiments performed to determine whether denaturation occurs within one minute, the length of time for which the

enzyme was kept at a denaturing temperature in the experiment cited above. The experiments summarized in Table II involve the use of highly concentrated enzyme solutions and of very short times of digestion. Therefore, the precision of these data is not to be compared with that of the other data in this paper.

In Table II (A), an enzyme concentration which gave an end point in 30 seconds at 40° C. gave an end point in the same length of time at 60° C., but at 50° C. the end point was reached in less than 15 seconds. The enzyme, therefore, was equally depressed from its maximum activity by the 10° deviation from the optimum and this depression occurred within 30 seconds. Furthermore, the denaturation must have occurred within 15 seconds or the end point for 60° C. would not have been prolonged beyond 15 seconds, the value for 50° C., and would in fact have been shorter due to the accelerating effect of the higher temperature. Table II (B) shows a decrease in activity of the order of 29 per cent when the temperature was changed from 50° to 60° C. for an amylase solution concentrated enough to give an end point in 5 seconds at 50° C. In C of Table II an increase in time to the end point from 15 to 25 seconds is recorded for the 10° temperature change.

TEMPERATURE °C.	A			B		C	
	40.	50.	60.	50.	60.	50.	60.
SECONDS TO END PT.	30.	<15.	30.	5.	7.	15.	25.
% INHIBITION			50.		29.		40.

Table II.—Time for the appearance of the reversible denaturation.

The data presented in Table III shows the effect on digestion time of adding the substrate a short time (15-60 seconds) after the enzyme solution is placed in a water bath at a temperature in the 57° to 60° C. range. In each case, addition of the substrate at 57° to 60° C. to the enzyme at the same time that the enzyme is subjected to the high temperature resulted in a reaction rate significantly greater than that obtained with an enzyme solution subjected to 57° to 60° C. for 30 to 60 seconds before the substrate was added. Only when the substrate was added within 15 seconds was there no alteration of rate.

The data presented in Table IV indicates that no digestion takes place within 15 seconds at 60° C. even for a digestion mixture which reaches an end point within 5 minutes at this temperature. In this experiment the 15-second sample of the digestion mixture was added to the standard iodine as usual, and the color of this sample was carefully compared visually with the standard iodine to which 1.0, 0.8, and 0.6 concentrations of standard starch had been added. No difference in color intensity between the 15-second digestion sample and the standard made with undiluted starch could be detected.

Tokuoka[1] states that prolonged heating of alpha amylase at 40° C. in the presence of Ca ion gave "perfect" protection against the inactivating effect of this temperature, while heating in the presence of Na ion gave no protective effect. The protective effect of Ca has been reported by many other workers. (Kneen, Sandstedt, and Hollenbeck,[7] Fukumoto[8]). The data presented in Fig. 2 shows that, in the case of salivary amylase, NaCl gives marked protection

EXPERIMENT	A	B	C	D
°C.	60.	59.	57.	60.
MIN. TO END PT. amylase-.25%NaCl starch	30.	11.	15.	19.
MIN. ALONE amylase-.25%NaCl no starch	0.5	1.0	1.0	0.25
MIN. TO END PT. starch added	65.	21.	24.	19.
% PROTECTION (approx.)	54.	48.	38.	0.

Table III.—The protection of amylase at high temperatures by substrate.

at 40° C. Experiments C and D in Table I indicate that the protective effect of NaCl extends to temperatures above the optimum where the denaturant effect occurs rapidly. In the presence of 0.25 per cent NaCl, no irreversible denaturation occurred within one minute at 60° C., while with 0.05 per cent NaCl the amylase was 38 per cent denatured irreversibly in one minute at 60° C.

SECONDS at 60°C.	15.	30.	60.	120.	240.	300.
COLOR	Blue (= cont)	Blue-violet	Dark violet	Violet	Violet-red	End point

Table IV.—The time course of a starch digestion by amylase as indicated by iodine color changes.

DISCUSSION

The optimum temperature for salivary amylase activity under the conditions outlined in Method is 50° C. That Bernfeld, Staub, and Fischer[9] found the optimum of crystalline salivary amylase to be 40° C. indicates the marked antidenaturant effect of impurities.

As the temperature is raised or lowered from the optimum, the activity of the enzyme is reversibly reduced. The rate at which the diminution in activity occurs differs on either side of the optimum temperature. At the extremes of the high and low ends of the temperature range the log of the activity varies in an essentially linear fashion with the reciprocal of the absolute temperature, and the heat of activation of the process dominant in each range may therefore be determined according to the method of Arrhenius. The heat of activation of the low temperature process is 22,742 calories, a value consistent with that for an enzyme-substrate reaction. The heat of activation for the high temperature process is 62,342 calories. A heat of activation of such magnitude

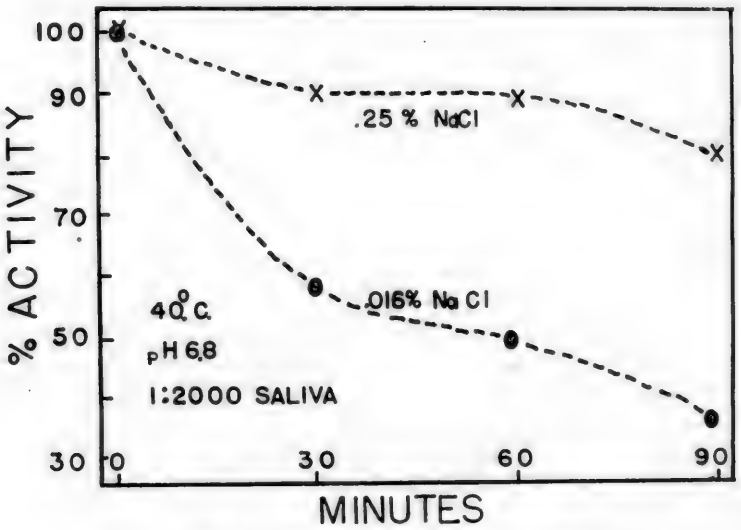


Fig. 2.—The protection of amylase by NaCl.

is not consistent with the values obtained for other enzyme-substrate reactions but is remarkably consistent with heats of activation for the reversible denaturation in bacterial luminescence (63,000 calories),[6] trypsin (67,600 calories),[2] and crystalline soybean trypsin inhibitor (55,000 calories).[10] It may, therefore, be inferred that the high temperature inactivation process is a denaturation. The data presented indicate that this process is reversible at one stage, and, therefore, salivary amylase, like the enzymes mentioned above, exists in solution in two forms: the active native amylase and the inactive reversibly denatured amylase. A third form, inactive irreversibly denatured amylase, may or may not be present depending on the conditions to which the enzyme has been subjected.

The reversible denaturation reaction for salivary amylase becomes appreciable within 15 seconds at 60° C. The enzyme-substrate reaction, on the other

hand, requires in excess of 15 seconds to become measurable at 60° C. under the conditions of enzyme and substrate concentration used in this study.

Tokuoka, in 1939, indicated that amylase may be protected against the irreversible inactivating effects of high temperature by a variety of substances including starch and its hydrolysis products but not NaCl. The data presented here indicates that NaCl serves as an excellent protective agent for salivary amylase. The findings of Tokuoka, in regard to the protective effect of substrate, however, have been confirmed and extended. We find that the presence of substrate protects the amylase against reversible denaturation. It is likely that the combination of native enzyme and substrate alters the equilibrium between native and reversibly denatured enzyme in favor of the native form due to the formation of a starch-enzyme complex and that the substrate exerts its protective effect in this manner. The fact that no protection is evident within 15 seconds and that this is also the time required for the appearance of digestion products further indicates the relationship between protection and the formation of the enzyme-substrate complex. In terms of the scheme outlined earlier, this mechanism may be presented as follows:

$$\text{Reversibly denatured enzyme} \underset{K_1}{\rightleftharpoons} \text{Native enzyme} \overset{\text{starch}}{\underset{K_2}{\rightleftharpoons}} \text{Native enzyme-starch complex}$$

The conditions of the experiments are, of course, critical. The enzyme without substrate was kept at 60° C. for a period not exceeding one minute. Within this time the enzyme is not irreversibly denatured. The enzyme is reversibly denatured within 15 seconds, and the enzyme-substrate reaction becomes appreciable only after 15 seconds. Consequently, it may be assumed that no appreciable digestion of starch occurred within the time for the reversible denaturation. It appears, therefore, that the greater rate of the enzyme-substrate reaction when the substrate is added to the enzyme, simultaneous to subjection of the enzyme to a denaturing temperature, is due to the protective effect of the starch, and that the mechanism of this protection is the effect that the chemical combination of the native enzyme and starch has on the equilibrium between native and reversibly denatured enzyme, that is, to cause a shift in the equilibrium in favor of the native form.

SUMMARY

The amylolytic activity of salivary amylase increases with a rise in temperature up to 50° C. As the temperature is raised beyond this optimum point, amylase activity drops off rapidly. The increase in activity with a rise in temperature up to 50° C. is due to the accelerating effect of increased temperature on the native amylase-starch reaction. The heat of activation for this reaction is 22,742 calories.

The decrease in amylase activity which accompanies a rise in temperature above 50° C. is due to inactivation of the amylase. The high heat of activation (62,342 calories) characterizing this process marks it as a denaturation.

One stage in the denaturation of salivary amylase is readily reversible. The denaturation process may be slowed by the presence of substrate and by NaCl.

It is a pleasure to acknowledge the help given by Drs. David Tanchester, Alexander Seelig, William Lefkowitz, and Mrs. Frances Greenfield in obtaining the equipment and supplies required for this study. I also wish to thank Dr. Lefkowitz for aid in preparing the manuscript.

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THE VALIDITY OF HAMSTER STUDIES IN CARIES CONTROL

HELMUT A. ZANDER, M.S., D.D.S., VINCENT F. LISANTI, D.M.D., AND FREDERIC R. SHIERE, M.S., D.D.S.

Tufts College Dental School, Boston, Mass.

INTRODUCTION

ALTHOUGH the Syrian hamster is widely used in caries research, there is no comparative study between hamster and human caries. This information seems essential to justify the use of the hamster in research directed toward human caries control. A comparison between experimental and human caries control is now possible in the light of investigations carried out with a dentifrice containing an antibiotic.

EXPERIMENTAL

1. *Penicillin.**—One hundred ten hamsters were divided into six groups with littermate controls. Each group was treated as follows:

Group 1. No brushing.

Group 2. Daily brushing with the base dentifrice.

Group 3. Daily brushing with the base dentifrice plus 62.5 units of penicillin.

Group 4. Daily brushing with the base dentifrice plus 125 units of penicillin.

Group 5. Daily brushing with the base dentifrice plus 250 units of penicillin.

Group 6. Daily brushing with the base dentifrice plus 500 units of penicillin.

The animals were placed on the experimental regime immediately after weaning and fed a caries-producing diet ad libitum.† All animals were sacrificed after 90 days, except for 6 animals lost during the course of the experiment. The results are shown in Table I and Fig. 1.

2. *Aureomycin.*—Eighty-four animals were divided into 3 groups and maintained as above. The 3 groups were treated as follows:

Group 1. Daily brushing with the base dentifrice.

Group 2. Daily brushing with the base dentifrice plus 0.1 mg. aureomycin per Gm. of dentifrice.

Group 3. Daily brushing with the base dentifrice plus 500 units of penicillin per Gm. of dentifrice.

All animals were sacrificed after 90 days, except for 6 animals of the penicillin group which escaped. The results are shown in Table II.

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*Activity of each dentifrice containing penicillin tested before and after experiment.

†Caries-producing diet:

Finely powdered cornstarch	56 per cent
Powdered whole milk	30 per cent
Powdered sugar	10 per cent
Alfalfa	3 per cent
Salt	1 per cent

TABLE I

RESULTS OF PENICILLIN STUDY

GROUP 1*			GROUP 2			GROUP 3			GROUP 4			GROUP 5			GROUP 6		
CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES
10	551	21	12	1,568	26	9	329	15	10	242	11	5	58	6	3	37	3
12	2,158	40	10	484	18	9	1,073	21	11	425	19	4	145	5	1	10	1
11	3,010	60	10	1,442	23	8	871	18	8	242	17	6	145	10	4	39	4
10	1,139	34	7	465	17	11	4,433	26	8	561	24	1	29	2	9	667	5
12	4,462	38	12	1,897	50	8	1,478	29	6	174	8	10	106	10	4	87	9
12	4,220	41	11	2,546	37	12	1,771	34	8	396	12	4	68	4	1	19	1
12	1,546	48	9	803	26	12	2,084	53	7	474	16	1	19	1	4	68	6
10	3,436	20	11	862	28	10	832	23	6	116	8	7	271	15	0	0	0
12	10,193	53	12	3,078	39	6	290	14	0	0	0	10	357	21	5	193	9
12	3,659	46	11	2,449	47	9	377	17	8	494	12	4	58	5	6	87	7
12	6,824	54	12	4,404	50	7	232	12	4	67	4	6	929	14	4	58	4
12	2,807	46	12	4,636	47	8	193	14	6	145	11	6	136	8	8	665	13
			12	5,004	52	4	48	5	7	203	14	5	174	9	3	48	5
			12	8,179	49	8	145	9	9	232	11	7	223	9	5	271	15
			12	2,042	37	7	194	14	5	794	8	4	57	4	5	66	6
			10	687	24	10	290	19				5	116	10	6	271	10
			12	2,701	27	7	271	19				7	513	11	5	145	8
			12	3,978	37	11	852	32				6	58	6	3	135	5
			12	1,317	29	8	271	16							5	48	5
						9	609	17							3	48	4
Total 137	44,005	501	211	48,542	663	173	16,643	407	103	4,565	175	98	3,462	150	84	2,962	120
Average 11.4†	3,667‡	41.8§	11.1	2,555	34.9	8.7	832	20.4	6.9	304	11.7	5.4	192	8.3	4.2	148	6.0

*Group 1. No brushing—12 hamsters.
Group 2. Base dentifrice—19 hamsters.
Group 3. Base dentifrice plus 62.5 units of penicillin—20 hamsters.
Group 4. Base dentifrice plus 125 units of penicillin—15 hamsters.
Group 5. Base dentifrice plus 250 units of penicillin—18 hamsters.
Group 6. Base dentifrice plus 500 units of penicillin—20 hamsters.

†Average number of affected teeth per animal.

‡Average surface area per animal.

§Average number of affected surfaces per animal.

TABLE II
RESULTS OF HAMSTER STUDY

GROUP 1*			GROUP 2			GROUP 3		
CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES
11	7,800	36	10	8,400	35	2	1,300	4
11	3,800	31	5	3,800	9	2	800	4
8	3,400	15	4	600	7	1	120	1
11	7,200	42	8	2,800	17	4	1,000	7
11	6,200	49	8	2,200	15	2	1,400	4
8	3,200	12	6	1,400	11	2	800	4
11	3,000	31	10	4,400	24	4	600	4
11	6,600	52	6	3,400	7	1	900	2
11	11,000	42	12	5,800	38	1	300	1
11	8,200	44	4	2,200	8	1	180	2
9	4,000	24	5	2,000	10	1	100	1
8	4,400	20	3	600	5	2	600	3
5	1,400	9	5	2,600	8	0	0	0
5	2,600	11	6	2,000	9	0	0	0
12	6,400	46	3	1,000	5	0	0	0
12	15,200	58	3	1,200	5	0	0	0
5	2,800	9	1	400	1	0	0	0
6	3,800	13	3	1,000	6	0	0	0
11	4,400	20	4	3,200	6	0	0	0
9	3,000	20	7	2,600	12	0	0	0
12	10,600	57	6	3,400	9	0	0	0
11	9,000	36	5	3,800	8	0	0	0
12	13,000	59	7	3,200	16			
8	2,600	26	5	2,600	10			
11	4,000	27	7	4,600	14			
9	1,200	12	4	1,000	4			
8	2,800	14	7	3,400	12			
12	3,800	46	2	600	2			
Total 269	155,400	861	156	74,200	313	23	8,100	37
Average 9.6†	5,500‡	30.8§	5.6	2,650	11.2	1.1	368	1.7

*Group 1. Control group—28 hamsters.
Group 2. Aureomycin group—28 hamsters.
Group 3. Penicillin group—22 hamsters.
†Average number of affected teeth per animal.
‡Average surface area per animal.
§Average number of affected surfaces per animal.

3. *Tyrothricin.*—One hundred thirty-two animals were divided into 6 groups and maintained as described previously. The 6 groups were tested as follows:

Group 1. No brushing.

Group 2. Daily brushing with the base dentifrice.

Group 3. Daily brushing with the base dentifrice plus 0.2 mg. tyrothricin per Gm. of dentifrice.

Group 4. Daily brushing with the base dentifrice plus 1.0 mg. tyrothricin per Gm. of dentifrice.

Group 5. Daily brushing with the base dentifrice plus 2.0 mg. tyrothricin per Gm. of dentifrice.

Group 6. Daily brushing with the base dentifrice plus 500 units of penicillin per Gm. of dentifrice. All animals were sacrificed after 90 days, except for 5 animals lost from the control and tyrothricin groups. The peni-

TABLE III

RESULTS OF TYROTHRICIN STUDY

GROUP 1			GROUP 2			GROUP 3			GROUP 4			GROUP 5			GROUP 6		
CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES	CARIOUS TEETH	AREA	SURFACES
2	125	3	3	350	5	0	0	0	0	0	0	10	175	3	0	0	0
2	240	4	7	490	11	1	50	2	2	80	3	3	175	7	0	0	0
4	1,270	17	7	490	13	3	80	4	1	80	2	3	375	7	0	0	0
7	1,525	13	5	545	9	4	175	5	11	80	1	7	500	15	0	0	0
9	2,400	28	4	810	17	3	225	5	7	325	10	9	545	18	0	0	0
8	2,400	17	8	920	18	6	330	10	5	590	9	5	590	8	0	0	0
8	2,400	17	6	950	17	9	340	5	9	800	19	5	590	10	0	0	0
9	2,800	20	8	1,500	17	6	490	12	7	800	31	6	745	15	0	0	0
4	2,850	13	6	1,750	16	4	625	8	10	1,500	20	7	1,155	16	0	0	0
10	3,770	29	7	2,000	18	6	690	14	9	1,610	24	11	1,270	25	0	0	0
12	3,940	37	7	2,000	18	7	1,040	22	12	1,610	29	9	1,500	22	0	0	0
7	4,550	18	10	2,260	26	11	1,250	28	10	1,830	22	9	1,725	28	0	0	0
10	4,550	26	7	2,850	17	9	1,725	31	9	2,400	29	11	1,940	28	0	0	0
12	5,025	36	9	3,050	22	6	1,835	16	9	2,600	24	7	2,150	18	0	0	0
11	5,490	39	11	3,170	29	7	2,150	21	10	3,490	33	11	2,400	27	2	125	3
11	6,500	38	10	3,250	24	9	3,050	21	12	3,490	44	9	2,840	27	2	175	4
12	6,675	43	9	3,450	22	11	4,250	39	12	4,600	45	11	4,490	35	3	275	6
11	7,800	34	10	3,490	28	10	5,250	38	10	4,875	30	12	4,875	47			
12	8,750	45	8	4,800	27	11	5,450	34	10	5,250	31	11	5,025	32			
12	9,350	35	10	5,700	34	11	6,500	39	10	6,150	36	12	5,135	51			
11	9,875	44	12	7,700	14	11	6,700	13	11	6,375	39	11	6,375	46			
						11	8,700	36	10	8,150	33	12	7,925	41			
						12	8,750	42				12	9,250	42			
Total 184	92,285	556	164	51,525	402	168	59,655	445	186	56,685	514	203	61,750	568	7	575	13
Average 8.8†	4,395‡	26.5§	7.8	2,454	19.1	7.3	2,594	19.4	8.5	2,577	23.4	8.8	2,685	24.7	0.4	34	0.8

*Group 1. Control group—21 hamsters.
Group 2. Base dentifrice 21 hamsters.
Group 3. Base dentifrice plus 0.2 mg. tyrothricin per Gm. of dentifrice—23 hamsters.
Group 4. Base dentifrice plus 1.0 mg. tyrothricin per Gm. of dentifrice—22 hamsters.
Group 5. Base dentifrice plus 2.0 mg. tyrothricin per Gm. of dentifrice—23 hamsters.
Group 6. Base dentifrice plus 500 units of penicillin per Gm. of dentifrice—17 hamsters.

†Average number of affected teeth per animal.

‡Average surface area per animal.

§Average number of affected surfaces per animal.

cillin group was short from the start due to littermate division. However, this was considered adequate due to previous results with the penicillin dentifrice. The results are shown in Table III.

DISCUSSION

The effect of the penicillin dentifrice (500 units per Gm.) upon the incidence of human caries has been reported in detail elsewhere.[1] After one year of brushing with the penicillin dentifrice, an over-all reduction of 50 per cent in new caries teeth and 55 per cent in new carious surfaces was noted; after 2 years, a reduction of 35 per cent in new carious teeth and 54 per cent in new carious surfaces.

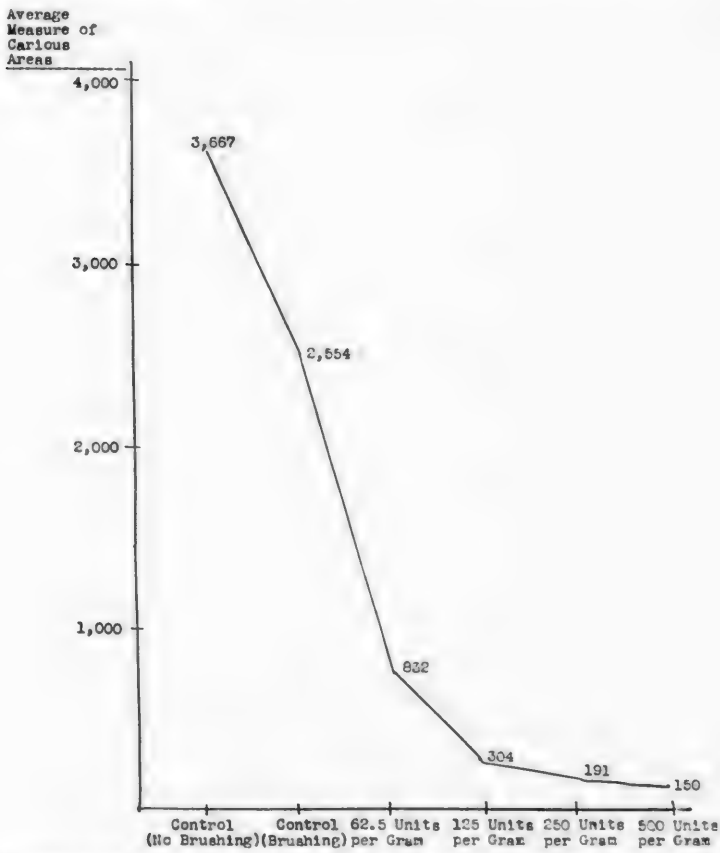

Fig. 1.—Hamster Test 2. Dentifrices containing different levels of penicillin. Average measure of carious areas.

In three consecutive hamster studies, a dentifrice containing 500 units of penicillin per Gm. produced reductions of 63 per cent in carious teeth and 94 per cent in carious area; 88 per cent reduction in carious teeth and 93 per cent

reduction in carious area; 95 per cent reduction in carious teeth and 98 per cent reduction in carious area. The consistency of the reduction in carious teeth and area is obvious.

The reductions in both human and hamster caries are highly significant. While the percentage inhibition of human caries by penicillin is somewhat less than in hamsters, this might be explained by the better control that can be maintained in the laboratory.

Whatever difference may exist between human and hamster caries, it has been established that the results from hamster caries-control studies were successful in predicting effectiveness in human studies. This would justify utilizing hamsters in caries research.

In addition, the above experiments show that there is an optimal concentration of penicillin for the control of hamster caries. There is probably an optimal concentration for human caries control, but not necessarily the same as for hamsters.

While aureomycin has some effect on hamster caries, it does not approach the efficacy of penicillin. Tyrothricin in the form used was ineffective in controlling hamster caries.

SUMMARY

Three antibiotics have been tested for their efficacy in reducing hamster caries. When applied in dentifrice form by brushing, penicillin concentrations of 500 units per Gm. were most effective, aureomycin had less efficacy, and tyrothricin was ineffective. The hamster findings with penicillin had a value in predicting effectiveness of a recent clinical study on human beings.

This is evidence to justify the use of hamsters in caries research.

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THE EFFECT OF COMPOSITION OF LIQUID AND POWDER ON THE PHYSICAL PROPERTIES OF SILICATE CEMENTS

R. S. MANLY, C. F. BAKER, P. N. MILLER, AND F. E. WELCH

Department of Chemistry, Tufts College, and Tufts College Dental School, Boston, Mass.

DENTAL silicate cements have been used extensively for anterior restorations because of their ability to match the color and translucency of teeth. These properties constitute just about their only superiority to dental amalgam. It has been reported that silicates have insufficient durability in the mouths of some persons[1] and that under some circumstances they have a deleterious action on the pulp.[2] The crushing strength of silicates is lower than that of dental amalgam[3] and it is generally recognized that the silicates are brittle and probably have a low impact strength.

The literature does not indicate that a thorough study has been made concerning possibilities of improvements. The first true silicous cement was described by Fletcher in 1871. It consisted of a powder component of fused alumina, silica, and calcium oxide for combination with a liquid containing tungstate and soda at first in gelatin solution and later in phosphoric acid.[4] In 1904 Steenbock marketed the Ascher cement which was stated as employing beryllium silicate for the powder, and a liquid consisting of a saturated H_3PO_4 solution of zinc, aluminum, and strontium phosphates.[5] The Ames silicate was described in 1909.[6]

In 1916 Voelker summarized the development of the silicates and gave such information as could be obtained concerning the 5 powders most widely used at that time.[7] He reported that the cements disintegrated in acid more rapidly than in water and presented the hypothesis that local acidity in the mouth might account for the lack of durability of cements. A relation between the mixing technic and the strength and setting time of the cement was demonstrated by Poetschke, who also investigated the effects of temperature, humidity, and powder to liquid ratio.[8] Ray gave data on composition of 3 brands of cement powders and studied the effect of acid concentration of liquid on the dimensional stability of the cement; a high acid concentration resulted in expansion and a low concentration produced a contraction.[9] Most of these studies have been summarized in the Bureau of Standards publication of dental materials.[10]

It was the purpose of this investigation to study modifications in the composition of both the liquid and powder components, with the goal of improving some of the properties of silicate restorations. Experimental powders were produced by varying the ratios of the 3 basic oxides, aluminum, calcium, and silicon, or the type and amount of flux in the fusion. Experimental liquids

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were obtained by changing the kind or amount of metal used as modifier in the aqueous phosphoric acid solution. All powders were mixed with a commercial liquid or its equivalent, and the physical properties of the set cement compared with those of one or more commercial cements. The experimental liquids were all mixed with a commercial powder and the results of the set cement compared with one or more controls made from commercial liquids and powders.

EXPERIMENTAL PROEDURE

Preparation of the Powders

All powders were prepared from Baker & Co.'s C.P. Silicic acid, C.P. hydrated alumina of the Aluminum Company of America, and Merck Reagent calcium carbonate. Fusion was carried out in a Sentry Globar furnace having an upper temperature limit of 1,538° C. (2,800° F.). Temperature was automatically controlled to ±50° F. with a Foxboro potentiometer. Compositions for fusion without flux were selected from a composition temperature phase diagram of CaO-SiO_2-Al_2O_3 system shown in Fig. 1 (Plate 2 prepared by Hoffman and Anderson of United States Steel). Ten gram samples were weighed out accurately on an analytical balance, and mixed thoroughly in a weighing bottle before being transferred to a platinum crucible. The first samples used were selected from the 4 eutectics bounding the anorthite range in the diagram. Other samples were fused within the 1,300° C. and 1,500° C. isotherms. In each of these samples the percentage of one of the oxides was kept constant with a previous sample. The tridymite range of the diagram was ruled out after preliminary results from samples of the adjacent area of the anorthite range lacked setting properties.

Each sample required from 1 to 3 hours to reach the temperature of from 1,370° to 1,540° C. and was held at this temperature from 3 to 8 hours. Whenever fusions produced glasses that were not clear, a second or third fusion was employed. The fused samples were quenched by immersing the platinum crucible under water with agitation until it cooled. The glasses were removed from the crucible, dried at room temperature, and left in the desiccator overnight. The glass was crushed repeatedly in a special steel mortar, prepared for us through the courtesy of the Carnegie Geophysical Laboratory, until it passed a No. 325 standard mesh bolting cloth. Iron particles were removed with a magnet.

Powders prepared with fluxes were made from CaO, Al_2O_3, and SiO_2 plus one of the fluxes—sodium carbonate, sodium fluoride, calcium fluoride, boric oxide, beryllium compounds, or metal phosphates. The ratio of oxides corresponded to that of the anorthite and mullite ranges. The mullite range was tested because most commercial powders have oxide ratios similar to mullite.

Preparation of Liquids

The first screening for selection of metals as experimental modifiers in the phosphoric acid liquid involved the elimination of those which form colored sulfides. There remained barium, beryllium, calcium, lithium, indium, magnesium, and titanium in addition to the zinc and aluminum that are commonly

employed. The solubilities of oxides, hydroxides, and carbonates of these metals were tried in phosphoric acid, in order to learn which forms might dissolve readily and sufficiently to permit the metal to have an influence on the phosphoric acid solution. Low solubility in phosphoric acid eliminated titanium oxide and cast doubt on the utility of calcium and barium oxide.

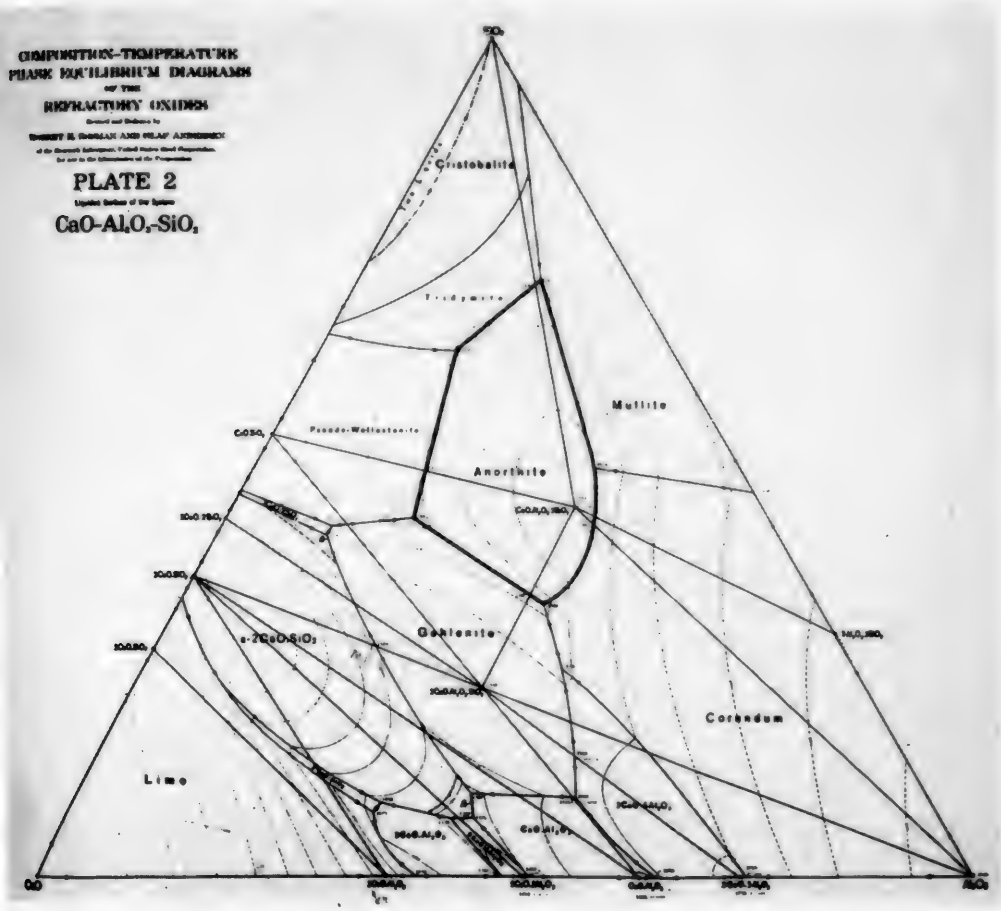

Fig. 1.

The experimental liquids were prepared by making the proper amount of oxide into a thin paste with part of the water required by the formula, and adding the total phosphoric acid slowly. The solids were dissolved with gentle heat, keeping the solutions below the boiling point. This solution was brought to final weight by the addition of water.

Tests on Physical Properties

Experimental powders were first tested qualitatively with S. S. White Dental Liquid in order to obtain evidence of setting properties. Our control

liquid consisted of 35 per cent P_2O_5, 4 per cent Al_2O_3, 6 per cent ZnO, and 54 per cent H_2O. Its composition was suggested by an approximate analysis of S. S. White Dental Liquid Filling Porcelain which it was intended to resemble in properties, but not necessarily in composition. If setting action were observed, a consistency test was next run in order to determine the powder required by 0.4 ml. of liquid.[11] All cements were mixed in a double-walled copper box having 3 sides and a bottom. It contained water for maintaining temperatures at 20° C. The top was covered with glass and the front with thin rubber sheeting through which the operators could perform the mixing operations while wearing rubber gloves. Liquids were stored in vaccine bottles and withdrawn and measured by 2 ml. hypodermic syringes. The remainder of the consistency tests were performed according to standard technics except that the specifications were increased to ±2 mm. instead of ±1 mm. because of difficulties in obtaining uniform results with this test.

The setting time was determined by the standard procedures[12] using a powder to liquid ratio as determined by the consistency test. The disks obtained from the consistency tests were used for the water solubility determination.[13] An indication of the strength of the set cement was obtained from indentation hardness tests with the Wilson Tukon Tester using a Knoop diamond indentor. This was employed rather than the usual crushing strength since the equipment was available. Five-tenths milliliter of the unset cement having the correct consistency was placed between 2 glass slides in a glass ring and was allowed to set for 1 hour in 100 per cent humidity at 37° C. The disks were then dried for 1 minute by a cold air blower and read on the machine. Five indentations were made across 1 diameter on both sides of the disk. Whenever possible, readings were taken where there was a clear surface with no bubbles or other imperfectations. The data represents the average of 10 readings on 1 cement disk with a load of 600 grams. The disks were placed in water for 1 week, and readings taken again. This permitted the hardness readings to be made on the samples used for the solubility tests.

On some of the experimental powders the refractive index was determined according to oblique illumination and Becke line method[14] using liquids ranging from 1.45 to 1.62 in refractive index. Tests on the translucency of some of the cements were carried out by placing 0.5 ml. of the unset cement of the correct consistency between 2 glass slides separated by a brass ring exactly 1 mm. thick. The cement was allowed to set for 1 hour at 100 per cent humidity at 37° C. The disks were kept immersed in water until ready for optical density readings with the Weston Health Protection densitometer.

Experimental liquids were studied by preparing cements from them with S. S. White powder No. 22 (after April, 1949, shade No. 23 was employed). Consistency, setting time, hardness, and water solubility were determined in the order mentioned. An attempt was made to develop a procedure for measuring the solubility of the cements in acid because of the possibility that such a method might parallel the disintegration of dental silicates in the mouths of certain persons. A gravimetric method, similar to the water solu-

bility tests, was eventually developed using a volatile buffer of ammonium acetate-acetic acid at pH 4. After a week in the buffer solution, disks were removed and the solution transferred to a platinum crucible, evaporated to dryness and ignited. The residue attributed to the buffer salts was less than 0.1 per cent. Tests with the control cement gave a value of 3.4 per cent solution in acid. During the development of the acid solubility test, a wet gravimetric method was tried. The disks were immersed in acid for a period of time, then removed, and blotted with a wet chamois. It was surprising to find that the disks constantly gained weight, suggesting that the disks were expanding and absorbing waer. A preliminary test on cement cylinders suggested an expansion of over 2 per cent after 1 month, but a more exact test of expansion over a period of 2 months indicated an expansion of only 0.2 per cent. This is within experimental error. An explanation of the weight increase in the acid cannot be offered at this time.

TABLE I

EFFECT OF VARIATION IN ALUMINUM AND ZINC CONTENT OF LIQUID MODIFIER ON PROPERTIES OF THE CEMENT

	S. S. WHITE		CONTROL		ALUMINUM		ZINC	
Liquid composition			(%)		(%)		(%)	
P_2O_5			35		35		35	
Al_2O_3			4		9		0	
ZnO			6		0		10.8	
H_2O			55		56		54.2	
Consistency: grams of powder, 0.4 ml. liquid, and disk diameter	(GM.)	(MM.)	(GM.)	(MM.)	(GM.)	(MM.)	(GM.)	(MM.)
	1.42	27	1.48	27	1.46	29	1.46	30
	1.44	30	1.46	26	1.49	25	1.46	27
	1.46	27	1.46	24	1.49	23	1.46	30
	1.46	26	1.46	24	1.49	27	1.50	25
	1.46	24					1.50	25
Setting time in minutes	(MIN.)		(MIN.)		(MIN.)		(MIN.)	
	5		3		3		3	
	4				3			
	3							
Water solubility in per cent	(%)		(%)		(%)		(%)	
	0.96	0.98	1.31	0.94	1.28	1.08	1.38	0.91
	1.01	1.12	1.03	0.96	1.25	0.93		
Knoop hardness	*1 hr.*	*1 wk.*	*1 hr.*	*1 wk.*	*1 hr.*	*1 wk.*	*1 hr.*	*1 wk.*
	50.6	61.1	53.9	54.5	51.6	63.4	56.5	77.0
	47.1	46.2	55.8	53.3	50.6	76.2	56.5	62.6
	51.0	53.9	54.5	51.7	57.6	66.8	57.9	68.5
	49.4	53.9	49.4	51.7	53.9	61.9	57.9	78.0
	55.8		57.2	51.7	54.5	66.7	55.2	61.9
			51.7	53.9	53.9	57.9	59.1	64.2
Average	50.8	53.8	53.8	52.7	53.7	64.5	57.2	68.7

RESULTS

Experimental Liquids

The first study was a comparison between a commercial liquid, the prepared control liquid, and liquids containing zinc or aluminum as a single modifier. The aluminum-modified liquid contained 9.03 per cent Al_2O_3, which was molecularly equivalent to the percentage of zinc it replaced in the control liquid. The results are presented in detail in Table I as an indication of the

precision of the tests. There was no major difference in the properties of cement prepared with these 4 different liquids. After 1 year's standing, the aluminum-modified liquid showed heavy precipitation, while the others exhibited only slight cloudiness. Perhaps the combination of aluminum and zinc together as a modifier avoids precipitation during storage, by maintaining each metal considerably below its saturation point in the phosphoric acid solution.

Magnesium oxide alone, or as a comodifier with zinc oxide, produced a liquid which would not set in the specified time limit when mixed with the control powder. The dilution of the liquid to hasten the setting time, produced chalky and brittle cements. Even when the cements were officially set, as judged by the Gilmore needle test, the disks were plastic and could be molded by finger pressure.

Metallic indium was dissolved in hydrochloric acid and precipitated as the hydroxide. A liquid containing only indium oxide produced a cement which had a dark porous surface and was softer than the control cement. A composition containing 2.3 per cent In_2O_3, 2.3 per cent ZnO, and 55.8 per cent H_3PO_4 produced a cement with a hardness equivalent to that of the control and a clear and shiny surface. Indium appeared satisfactory as a comodifier but there was no indication of improvement in properties.

Calcium oxide was found to be soluble to the extent of 1 per cent in the aqueous phosphoric acid employed. Higher percentages dissolved upon heating but reprecipitated upon cooling. About 8 per cent ZnO could be incorporated with CaO as a comodifier. Only 4 per cent Al_2O_3 could be incorporated along with 1 per cent CaO. Cements prepared from liquids with zinc and calcium, or aluminum and calcium, as comodifiers, were approximately equivalent in hardness and water solubility to cements prepared from the control liquids.

The behavior of barium oxide as a modifier was similar to that of calcium oxide. At the high acid-to-water ratio which was required to lengthen the setting time to 3 minutes, only 3 per cent of the oxide could be dissolved. Water solubility and hardness tests on cements containing either the BaO liquids or a BaO-ZnO liquid gave results no better than the control liquid.

Liquids modified with beryllium oxides were very viscous and required considerable dilution. The oxides dissolved only after long heating when most of the water had been driven off. The proper acid concentration for the beryllium liquid appeared to be about 64 per cent, but this may be in error because both acid and water were probably lost during evaporation. The beryllium-modified liquids produced cements which were only 0.7 per cent soluble in comparison with 1.0 per cent for the control. They likewise exhibited a property of delayed hardening. The hardness was low 1 hour after mixing and increased by 12 to 30 units by the end of 1 week. At this time the surfaces of the cements were much harder than the controls. Both of these points of superiority constitute important improvements, but it is questionable whether the BeO liquids deserve further investigation in view of the recent evidence on the high toxicity of beryllium compounds.

In these studies all comparisons were with one manufacturer's product. As a check on the representativeness of this product, it was compared with two other commercial silicates. The data in Table II indicate that the product selected is representative and approximately equivalent to the others commonly available.

The Ternary System, SiO_2-Al_2O_3-CaO

The anorthite range of this 3-component system is bounded by 4 eutectics, whose melting points lie between 1,165° and 1,382° C. (Fig. 2). Samples A, B, C, and E correspond to these eutectics in composition. The remaining 16 samples either lie along the boundary lines connecting the eutectics or at other sites which would be expected to melt at less than 1,500° C. In most instances, compositions not on a boundary have a percentage of one of the oxides that is the same as one of the boundary samples. Nineteen samples lie within the anorthite range and one in the pseudo-wollastonite range.

TABLE II
PHYSICAL PROPERTIES OF THREE COMMERCIAL DENTAL SILICATES

MANUFACTURER	CONSISTENCY (GM. OF POWDER)	SETTING TIME (MIN.)	H_2O SOLUBILITY (%)	HARDNESS	
				1 HR	1 WK.
The S. S. White Dental Mfg. Co.	1.52	4	1.02	54 57 60	55 58 56
Lee and Smith	1.46	5	1.03	46 44 46	42 46 53
L. D. Caulk Co.	1.62	4	0.80	44 48	40 43

All of the compositions fused to clear or opaque glasses. Seven of these exhibited no setting action with silicate liquid. Spatulation with liquid merely produced a friable mass of moist powder. The nonsetting compositions occurred in the high silica area of the anorthite range with SiO_2-CaO ratios of approximately 2.08 or greater. Some compositions in the tridymite range fuse below 1,500° C., but these were not studied because of their high silica content. Additional fusions with Samples B, E, O, and T failed to cause the compositions to set.

The slow-setting compositions occupy a narrow band just below the nonsetting range. Their composition approximately corresponds to a range of alumina from 11 to 28 per cent in a composition with SiO_2-CaO ratio of about 1.95. Powders I, L, and N are representatives of this region with setting times from 11 to 18 minutes, and low Knoop hardness from 9.5 to 17.2.

Moderately rapid setting was obtained with Samples M, P, and Q, which have somewhat lower SiO_2-CaO ratio than the slow-setting compositions. The samples occupy a narrow band just below the slow-setting group. The band has a SiO_2-CaO ratio of approximately 1.83 ± .06 and an Al_2O_3 content of 16 to 30 per cent. When the alumina content is high, the lime content must be slightly reduced, indicating that the alumina may substitute partially for lime.

TABLE III

PREPARATION DATA ON SILICATE POWDERS

NO.	FLUX	SiO_2 (%)	Al_2O_3 (%)	CaO (%)	FLUX (%)	FUSION NO.	FUSION NO. OF °C.	FUSION HOURS	APPEARANCE	SETTING SPEED
A	None	31.8	39.0	29.2	0	1	1426	5.5	Glass	Fast
B	None	70.4	19.8	9.8	0	2	1482	11.0	Glass	None
C	None	42.0	20.0	38.0	0	1	1426	5.0	Glass	Fast
D	None	36.9	29.5	33.6	0	1	1426	5.0	Glass	Fast
E	None	62.0	14.7	23.3	0	2	1426	10.0	Glass	None
F	None	52.0	17.4	30.6	0	1	1426	6.0	Glass	Fast
G	None	57.0	17.4	30.6	0	1	1426	5.0	Glass	None
H	None	62.0	17.4	20.6	0	1	1426	5.0	Glass	None
I	None	57.0	12.4	30.6	0	1	1426	5.0	Glass	Slow
J	None	46.4	22.8	30.6	0	1	1460	6.0	Glass	Fast
K	None	36.9	32.5	30.6	0	1	1520	6.0	Glass	Fast
L	None	52.0	22.8	25.2	0	1	1482	5.0	Glass	Slow
M	None	49.3	22.8	27.9	0	1	1482	5.0	Glass	Mod.
N	None	54.5	17.4	28.1	0	1	1482	5.0	Glass	Slow
O	None	54.5	20.3	25.2	0	2	1482	10.0	Glass	None
P	None	52.0	20.0	28.0	0	1	1516	5.0	Glass	Mod.
Q	None	49.3	25.5	25.2	0	1	1516	5.0	Opaque	Mod.
R	None	46.5	25.5	28.0	0	1	1516	5.0	Opaque	Fast
S	None	55.0	24.0	21.0	0	1	1516	5.0	Opaque	None
T	None	65.0	20.0	15.0	0	4	1482	20.0	Glass	None
3	Na_2O	57.0	13.4	21.6	8.0	1	1482	6.0	Glass	None
4a	CaF_2	49.5	11.8	18.7	20.0	2	1398	13.0	Glass	None
4b	CaF_2	49.5	11.8	18.7	20.0	1	1260	4.5	Opaque	None
5	CaF_2	39.7	28.0		11.3	1	1427	5.5	Opaque	Fast
	NaF				21.0					
6	Na_2O	39.7	28.0	11.3	21.0	1	1427	4.5	Glass	Fast
7	CaF_2	39.7	28.0	11.3	21.0	2	1482	12.0	Opaque	Mod.
8	CaF_2	44.3	10.7	16.4	14.3	1	1371	4.0	Glass	Fast
	Na_2O				14.3					
9	BeO	42.9	21.4	7.1	28.6	5	1538	23.0	Sinter	None
10	BeO	65.0	20.0	10.0	5.0	2	1538	9.0	Opaque	None
11	BeO	65.0	25.0	5.0	10.0	1	1538	4.0	Sinter	None
12	BeO	65.0	20.0	12.5	2.5	2	1538	9.0	Opaque	None
13	Beryl	50.4	19.4	27.2	3.0	1	1516	5.5	Glass	Mod.
14	Beryl	56.1	17.1	24.1	2.7	1	1516	5.5	Glass	None
15	NaCl	39.7	28.0	11.3	21.0	3	1537	20.0	Opaque	Slow
16	NaCl	39.7	28.0	17.3	15.0	3	1537	15.0	Opaque	Mod.
17	NaCl	39.7	28.0	22.3	10.0	4	1537	15.0	Opaque	Fast
18	NaCl	39.7	28.0	2.3	30.0	3	1537	13.0	Opaque	None
19	B_2O_3	52.0	14.7	23.3	10.0	2	1460	10.0	Glass	None
20	B_2O_3	42.0	14.7	23.3	20.0	1	1460	6.0	Glass	Mod.
21	B_2O_3	47.0	14.7	23.3	15.0	1	1427	4.0	Glass	Slow
22	B_2O_3	37.0	14.7	23.3	25.0	1	1482	4.0	Glass	Mod.
23	B_2O_3	39.7	28.0	11.3	15.0	1	1538	6.0	Opaque	Slow
24	B_2O_3	50.1	24.4	15.5	10.0	1	1482	6.0	Glass	None
25	B_2O_3	46.8	25.2	18.0	10.0	1	1482	6.0	Glass	Slow
26	B_2O_3	45.5	27.2	18.3	10.0	1	1482	5.0	Opaque	Slow
D1	P_2O_5*	35.6	28.6	33.4	2.4	1	1482	5.0	Glass	Fast
D2	P_2O_5*	34.6	27.6	33.1	4.7	1	1482	5.0	Glass	Fast
D3	P_2O_5†	36.0	28.8	32.8	2.4	1	1482	5.0	Glass	Fast
D4	P_2O_5†	35.1	28.0	32.0	4.8	1	1482	5.0	Glass	Fast
D5	P_2O_5‡	35.3	28.2	31.7	4.8	1	1482	5.0	Opaque	Fast
D6	P_2O_5‡	36.0	28.8	32.8	2.4	1	1482	5.0	Glass	Fast
P1	P_2O_5*	50.8	19.5	27.3	2.4	1	1482	6.0	Glass	Mod.
P2	P_2O_5*	49.5	19.0	26.7	4.8	1	1482	6.0	Glass	Mod.

*In form of $Ca_2P_2O_7$.
†In form of $AlPO_4$.
‡In form of $Ca_3(PO_4)_2$.

The moderate setting compositions possessed the best physical properties. The samples melted to clear glasses with 1 fusion at 1,482° C. M and P were clear and Q was opaque. From 1.00 to 1.20 Gm. powder could be incorporated in the consistency test. Disks were 28 to 34 units softer than control. The water solubility was 3.0 to 4.2 per cent which was lower than most other experimental powders but still higher than specifications.

The fast-setting compositions had SiO_2-CaO ratios lower than 1.74, and were located in the lower third of the anorthite range. Representatives from the 6 samples of this group had low powder to liquid ratios, low hardness, and considerable water solubility. Sample A was tried with an MgO-modified experimental liquid in hopes of extending the setting time, but the setting time was scarcely affected, and the disks were softer than with control liquid.

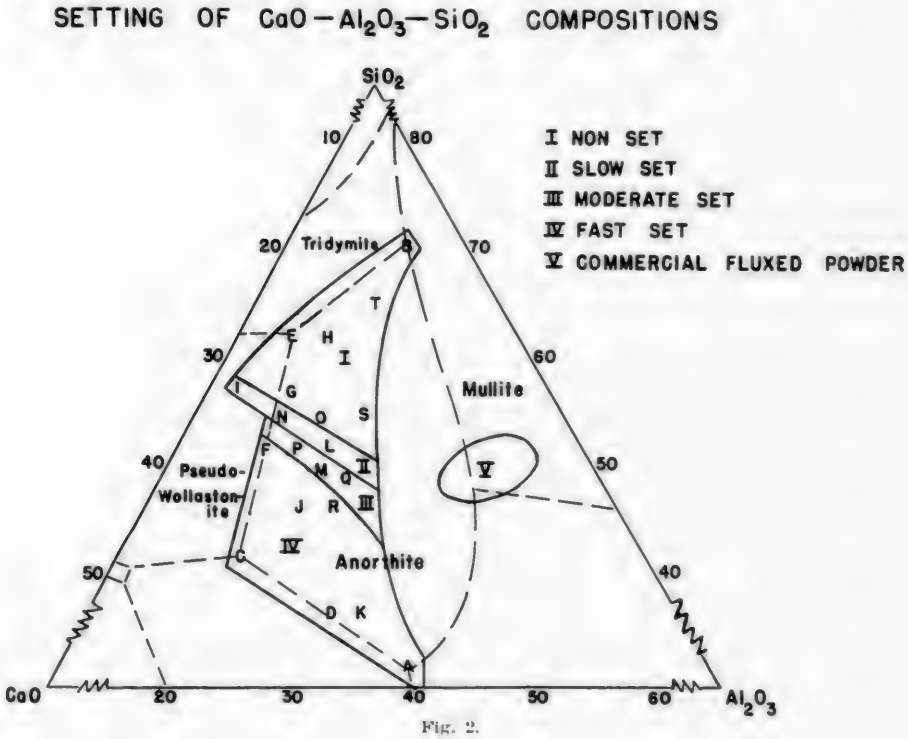

Fig. 2.

Silicate Powders Prepared With Flux

Seven different compounds were tested for suitability as fluxes. These included sodium carbonate and fluoride, sodium and calcium fluoride, beryllium compounds, boric oxide, and metal phosphates. The compositions tested and results obtained are presented in Table III.

Data on commercial silicate powders indicate that their CaO:SiO_2:Al_2O_3 ratio would correspond to the mullite range of the ternary system. For this

reason some of the compositions with flux were prepared with the mullite ratio of the oxides, and the remainder had oxide ratios corresponding to the anorthite range.

Eight compositions were tested with sodium carbonate, sodium fluoride, or calcium fluoride as fluxes. Sample 7 was the most satisfactory. Its ratio of oxides corresponds to commercial silicate powders and its CaF_2 content was 21 per cent. The hardness was lower than the control, but the water solubility was lower than any other composition tested and was within specifications. Nonsetting compositions were converted to rapid setting powders when sufficient flux was used. It thus appeared that addition of flux would cause cementing actions in a powder of high silicate content.

Sodium chloride was tried as a flux because of its chemical similarity to sodium fluoride. Four samples having NaCl percentages ranging from 10 to 30 per cent fused to form opaque glasses having a smooth marble-like surface. Tests with 2 of these (Samples 16 and 17) demonstrated low hardness and high solubility. Their low strength is probably attributable to the small amount of powder (0.75 to 0.85 Gm.) that could be incorporated into the liquid.

Several compounds of beryllium were tried as fluxes. Compositions 9 to 12, inclusive, were trials with BeO as flux. These compositions either failed to fuse or to exhibit cementing properties. An attempt was made to synthesize chrysoberyl, $BeO\text{-}Al_2O_3$, by heating 19.7 per cent BeO and 80.3 per cent Al_2O_3 for 12 hours at 1,540° C. Fusion failed to take place. The mineral beryl, $BeO\text{-}Al_2O_3\text{-}SiO_2$, was used as flux for Samples 13 and 14. Powder 14 failed to set, probably because of a slightly higher silicate content and Powder 13 showed substandard properties.

Purified boric anhydride, B_2O_3, was next investigated as a flux, at concentrations of 10, 15, 20, and 25 per cent, with partial substitution for the silica in Composition 1. Sample 19 contained only 10 per cent B_2O_3 and failed to give a setting action. Higher concentrations set at speeds roughly paralleling the flux content. The next 4 compositions had less CaO and more Al_2O_3 approaching the mullite range and approximating the composition of commercial silicate powders. None of these had sufficiently rapid setting action. The 2 borate-fluxed powders which were suitable for further test were Samples 20 and 22. Their hardness was low, their solubility high, and the surface of the cement was affected by 1 week's contact with water.

Phosphates were tried as fluxes for addition to Samples D and P. The compounds tested were calcium pyrophosphate, tricalcium phosphate, and aluminum phosphate at concentrations of 2.5 and 5 per cent of P_2O_5. When added to Sample D, a fast-setting powder, the reaction was still rapid and the cements were very soft. The addition of 2.5 and 5 per cent P_2O_5 as calcium pyrophosphate to the moderate setting powder lengthened the setting time considerably. Tests on consistency, setting time, hardness and water solubility gave inferior results. It appears that phosphates decrease the setting time but affect the other physical properties unfavorably.

Several of the better compositions were subjected to additional physical tests (Table IV). The index of refraction of the particles were compared with that of the control commercial powder. The range of n_D was from 1.51 to 1.61 with a mode at 1.58. The refractive index of the control powder was 1.47. Since it is probable that the matrix has a low refractive index, one would expect that the experimental powders would generally be more opaque than the control. The optical density (log Io/I) ranged from 2.31 to 2.60 which is much higher than that of the control, 1.90. The effect of additional fusions was determined by a study of sample P. Three 10-Gm. samples of this composition were prepared and given 1, 3, and 5 fusions respectively. There was little difference in the setting time of the 3 powders. The consistency test showed more powder could be incorporated into the liquids after additional fusions. Three or more fusions caused an increase of 17 per cent in hardness and lower translucency of the cements.

TABLE IV

TESTING DATA ON SILICATE POWDERS

NO.	FLUX	CONSISTENCY (GM.)	SETTING TIME (MIN.)	HARDNESS 1 HR.	HARDNESS 1 WK.	H_2O SOL.	POWDER N_D	OPTICAL DENSITY
A	None	0.70	4	10.0		5.9	1.59	
A	None	0.85	2-3	15.6				
A	None	1.00	6	14.7				
F	None	0.80	3	14.3			1.59	
I	None	1.14	18	11.4			1.59	
L	None	1.14	18	17.2			1.58	
M	None	1.05	5-6	18.1	18.3	3.9	1.59	
				18.3	19.3			
N	None						1.58	2.45
P	None	1.20	4-5	19.1	21.7	3.1	1.58	2.35
				17.9	21.9			
Q	None	1.20	6	19.8	19.0	4.2	1.58	
				19.8	18.7			
7	CaF_2	0.90	4	11.7	24.1	2.0	1.58	
13	Beryl	1.23	5-6	14.8	13.8	3.4	1.59	
				15.9	15.1			
16	NaCl	0.85	4	10.9	12.0	5.3	1.59	
				11.2	12.5	7.0		
17	NaCl	0.75	2-3	13.9			1.59	
20	B_2O_3	1.00	10-12	23.0	19.2	5.6	1.56	2.60
				19.5	15.9			
22	B_2O_3	1.01	9	20.8	16.3	5.4	1.57	
				21.5	17.7			
P1	P_2O_5	1.15	23	9.5	11.3	4.8	1.58	
P2	P_2O_5	1.15	25	6.3	8.5	6.5	1.58	2.40
Control		1.45(5)	4(3)	50.8	53.8	1.0	1.48	1.90

SUMMARY

A survey has been made of the possibilities of improving the properties of dental silicates by modification of the kind or amount of modifier in liquid and by varying the kind and amount of flux and the proportions of usual oxides in the powder. The suitability of various compositions was judged from determination of consistency, setting time, hardness, and water solubility. In some instances the acid solubility, the refractive index of the

powder, and the opacity of the set cement were measured. Liquids were evaluated by mixing these with a commercially available powder, and powders were compared in most instances after forming cements with a commercial liquid.

When aluminum or zinc was used alone as a modifier, the physical properties of the cement were approximately the same as that prepared from a special control liquid and that prepared from a commercial liquid. Magnesium, indium, calcium, barium, titanium, and beryllium proved to be unsuitable as single modifiers in the liquid. Barium, calcium, and indium oxides could be used as comodifiers with zinc oxide without deleterious effect on the physical properties, but there was no indication of general improvement.

Only a very narrow range of composition containing CaO, SiO_2, and Al_2O_3 could be fused to clear glasses which would set within the proper length of time with silicate liquids. Several different compounds could be used as fluxes for increasing the range of compositions that would set. No composition was found which was appreciably superior in properties to representative commercial products and all powders tested were deficient in translucency.

ADDENDUM

Since submission of this manuscript for publication, tests have been completed by Dr. Robert L. Glass on reaction of pulp of dog teeth to silicate powder samples P, 16, 20, and a control (S. S. White Dental Filling Porcelain). These powders were mixed with S. S. White Silicate Liquid. Three teeth were utilized for each sample in one dog sacrificed after 5 weeks and in another, sacrificed after 6 weeks. Histologic sections of the teeth in each of the 4 groups disclosed slight chronic inflammation of the pulp and irregular dentin formation beneath the cavity preparation. Since powder P contained no flux and 16 and 20 were made with chloride and borate flux, this finding suggests that the presence or type of flux in the powder is not the sole causative agent for pulpal irritation. (Received Feb. 1, 1951.)

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